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## ERRATA

Page 110, line 6 from bottom,  $\frac{-7.26a}{A}$  should read  $\frac{-0.26a}{A}$ .

Page 125, line 3, "c" should read "C".

Page 424, line 5 from bottom, "D" should read "E."

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NO. 1

## A NEW BACTERIAL CITRUS DISEASE<sup>1</sup>

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### INTRODUCTION

During the last few years a disease of Citrus trees, and particularly of orange trees, has been brought repeatedly to the attention of different members of the Agricultural Experiment Station staff of the University of California. In some respects the trouble resembles frost injury, so that many growers, believing that it was due to frost, did not appreciate its full significance. Occasionally material has been brought to the laboratories of plant pathology at Berkeley and at Riverside; but such material has been old and dried and has given no clue to the real cause.

In March, 1916, Dr. J. E. Coit, of the division of citriculture, called attention to this disease and discussed its seriousness.<sup>3</sup> Since he found no similar disease described in literature, he called it "Citrus blast," a new disease.

### DESCRIPTION OF THE DISEASE

During the rainy season of California, usually about the middle of January, the disease is first noticed. Young leaves are found to be dropping off, sometimes leaving single twigs or at other times whole branches of twigs bare of leaves. On examining more closely, black, discolored areas are noted on the leaves; such areas are found most commonly at the junction of the leaf blade and the wings of the petiole. Plate A, figure 3, shows such lesions. The affected parts have a water-soaked appearance, and the whole leaf loses its rigidity and hangs limply from the branch. Less commonly such water-soaked lesions appear near the tips of the leaves.

<sup>1</sup> Approved for publication by Thomas F. Hunt, Director, California Agricultural Experiment Station.

<sup>2</sup> Now Scientific Assistant, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture.

The author wishes to express appreciation and thanks to Prof. Ralph E. Smith, of the University of California, for direction of this work; also to Dr. William A. Setchell, of the same university, for the translation into Latin of the description of the organism.

<sup>3</sup> Coit, J. E. Citrus blast—a new disease in California. *In Univ. Cal. Jour. Agr.*, v. 3, no. 6, p. 234-235, illus. 1916.

The blackened areas frequently spread down the petioles of the leaves into the twigs upon which the leaves are borne. If such a twig is young and actively growing, the diseased area spreads quickly and the whole twig becomes blackened and shriveled. In young orchards, where there is much new succulent growth, these blackened, shriveled twigs, bare of leaves, are very common; and in this condition there is little similarity between such affected parts and frost injuries. The disease has never been found spreading down into the mature wood.

In March there are periods of several days in which the weather becomes very warm and the orchards dry up to some extent. Such weather causes the affected tissues at the base of the leaf blade and the affected leaf petioles to dry up, become stiff and rigid, and the leaves to hang to the tree. The trees in such cases resemble pear trees badly affected with pearblight in the young succulent twigs (Pl. 2).

In many cases the lesion spreads from the petiole of a leaf down into twig tissue which is still soft but not actively growing, and a small, black area around the base of the petiole is formed. After the leaf has dropped off and the dry season comes on, brown blister-like scabs are formed over such affected areas (Pl. A, 1, 2). As the twig continues growing, these scabs become loose and may be cast off during the succeeding fall. On trees which have been affected in the spring, however, such blisters are frequently found in the following winter, and these are presumably the sources for the dissemination of the disease.

#### DISTRIBUTION OF THE DISEASE

The disease was first observed in 1912 at Oroville, Cal., by Mr. R. W. Hodgson, of the University of California. Since then specimens have been received from time to time from the Oroville and Orland regions, and it has now been seen or reported in almost all the Citrus-growing regions of northern and central California, both on the orange (*Citrus aurantium* L.) and the lemon (*Citrus limonum* Risso). It has not been observed in southern California, and, so far as is known, no one has reported it from there as yet.

#### ISOLATION AND IDENTIFICATION OF THE ORGANISM

On January 24, 1916, fresh material of Citrus blast was sent in from Palermo, Cal. Sections through the lesions were made, and the tissues were seen to be filled with motile bacterial organisms. Isolations made from this fresh material in +10 standard peptone-beef bouillon<sup>1</sup> produced a clouding on the second day; on plating, these yielded several sorts of colonies. One of these types of colonies, on being inoculated into orange trees in the greenhouse, gave positive results con-

<sup>1</sup> All references to acidity are expressed in terms of Fuller's scale.

sistently; and reisolations from these inoculations gave the same typical colonies. The organism has been repeatedly inoculated and reisolated since that time, and has also been repeatedly isolated from material from different regions of the State, both from the fresh black lesions and the reddish blister-like scabs found in summer. Both types of lesions, the black watery effect on the leaves and twigs and the reddish scabs, have been produced from artificial inoculations with pure cultures. In the inoculation work young lemon and navel-orange trees have been used and kept in the greenhouse at temperatures of 20° to 25° C.

According to the methods in use at present by bacteriologists, the organism is a distinct species; and, so far as is known, has never been described in the literature on the subject. Owing to the withering or drying up of the leaves of Citrus trees following the attacks of this organism the specific name "*citrarefaciens*" is suggested—a compound of the two words "Citrus" and "arefacio." The brief Latin diagnosis is as follows:

**Bacterium citrarefaciens, sp. nov.**

Baculis cylindricis, apicibus rotundatis, solitariis aut interdum geminis,  $1.2-3 \times 0.4-0.9 \mu$ , vulgo  $0.6 \times 1.8 \mu$ , motilibus, flagellis 1-4, uni-aut-bipolaribus; methodo Grami non coloratis; cum acidibus decoloratis; sporis capsulisque nondum visis; zoogloeis defectis; statubus involutis, longe-filamentosis, massulas protoplasmaticas densiore tinetas complectentibus, coloniis in agar-agar orbicularibus, convexis, nitentibus, margaritaceo pallidis, colorem fluorescenti-viridem in medium alibile efficientibus; gelatinis primum celeriter deinde lente liquefacientibus; casein segregantibus; lactem litmus lente decolorantibus; in mediis saccharatis neque gas neque acidum evolvens; nitrum non reducentibus; indol ammoniamque moderate producentibus; aerobicis sed in presentia sacchari, sacchari urvae, aut sacchari hordei facultative anaerobicis. In textis vivis arborum citrinorum, laesiones foliorum cauliumque juvenorum, primum nigras deinde in mensibus siccis aestivalibus rubras scabrosasque efficiens.

#### HISTOLOGY

Affected areas of young leaves were fixed, embedded, sectioned, and stained. It was found that the organism in tissues could be stained best with Mallory's chlorid-of-iron hematoxylin, as described by Mallory and Wright.<sup>1</sup> Impregnation with gold chlorid, according to Lee,<sup>2</sup> also gave good results, the impregnation being carried on after the sections had been fixed on the slides, however, instead of *in toto*, as described by Lee.

Masses of bacteria could be observed throughout the parenchyma of the leaf, both in the cells and in the intercellular spaces. In many lesions all cell structures had disappeared, the areas being occupied by masses of the organisms (Pl. 1, C). The disease is not to be considered as an invasion of the vascular bundles, but rather as an attack upon the parenchyma.

<sup>1</sup> Mallory, F. B., and Wright, J. H. Pathological Technique . . . ed. 3, 469 p., 138 fig. Philadelphia, 1904.

<sup>2</sup> Lee, A. B. Microtometist's Vade-Mecum . . . ed. 7, 526 p., illus. Philadelphia, 1913.

## MORPHOLOGY AND PHYSIOLOGY OF BACTERIUM CITRAREFACIENS

## MORPHOLOGICAL CHARACTERS

When the organism is taken from 24-hour-old agar stroke cultures it is rod-shaped, with rounded ends, usually single, but occasionally in pairs. The limits of size are 1.2 to 3.0 by 0.3 to 0.9  $\mu$ , the most common size being 1.8 by 0.6  $\mu$ . No spores or capsules have been observed; zoogloea are not found; motility has been observed in affected tissue and from young cultures, one to four flagella at one or both poles having been shown from 24-hour agar cultures. Involution forms, long filament-like bodies in which there are thickened masses of protoplasm that take stains more heavily, are found in old cultures and especially in saccharose bouillon (Pl. 1, B).

The bacterial rods may be readily stained by watery-fuchsin, gentian-violet, carbol-fuchsin, and Löffler's alkaline methylene-blue. When stained lightly, a bipolar effect is brought out in the organism, the ends of the rods taking the stain more densely, leaving a lighter area in the middle. The organism is Gram-negative, and is not acid-fast.

## CULTURAL CHARACTERS

**AGAR POURED PLATES.**—On +10 peptone-beef agar at 20° to 22° C., surface colonies are apparent on the third day; on the fourth day colonies are 2 to 3 mm. in diameter, white, round, smooth, glistening, convex, finely granular (under the compound microscope), with entire edges sometimes becoming lacerate, due to the semifluid consistency of the bacterial mass. When 5 days old, the agar beneath the colonies becomes greenish and fluorescent, and in 7 days the colonies lose their convex glistening surface and dry down, frequently becoming concentrically ringed and the edges undulate to lobate. Buried colonies are biconvex.

**AGAR STABS.**—Stabs in +10 peptone-beef agar when 13 days old show growth along the line of inoculation to four-fifths of the total depth of the stab. Growth is best toward the surface, the line of puncture beaded; there is no liquefaction; surface growth is restricted. The agar medium is uniformly colored a javal-green<sup>1</sup> when seen against a black background.

**AGAR SLANTS.**—On slant agar, stroke cultures make an abundant growth in two days; filiform becoming somewhat echinulate, convex, glistening, smooth, translucent, of slimy consistency, and with a slight odor of putrefaction. A cream-white sediment forms in the V; the medium is colored uniformly a fluorescent-green.

**NUTRIENT-GELATIN PLATES.**—Growth rapid, at first punctiform, then round, crateriform; liquefaction spreading.

**GELATIN STABS.**—At 18° to 19° C., in +15 peptone gelatin, liquefaction starts on the first day, is crateriform until on the fourth day the pit of liquefaction reaches the walls of the tubes when it becomes stratiform. The liquefaction gradually slows up and takes 60 days or more to reach completion.

**BEEF BOUILLON.**—In +10 peptone-beef bouillon a moderate uniform clouding appears within 24 hours; there is no turbidity. At first there is formed a very thin pellicle which disappears within a few days. Old cultures contain a sediment which is very slightly viscid. There is a slight odor of putrefaction.

<sup>1</sup> All references to color are expressed according to Ridgway (Ridgway, Robert, *Color Standards and Color Nomenclature*. 43 p., 53 pl. Washington, D. C., 1912).



**POTATO CYLINDERS.**—Growth on potato cylinders is moderate, filiform, becoming echinulate, and spreading with age; at first convex, becoming flattened, glistening, smooth, cream-buff in color, the medium itself being slowly browned until it becomes avellaneous. There is a slightly saline odor from the cultured cylinders. At the end of the fifteenth day starch reduction was tested by means of iodine in a potassium-iodid solution. Uninoculated cylinders gave the typical blue starch reaction, but cultured media gave the port-wine color indicative of the formation of amylopectin or still lower reduction products.

**MILK.**—The casein in inoculated milk coagulates slowly, leaving at the surface a clear liquid which has a fluorescent-green color. Peptonization of the coagulum then takes place slowly, being complete in 25 to 30 days. The consistency of the medium is unchanged.

**LITMUS MILK.**—Growth in litmus milk is distinctive; the coagulated portion remains the original lavender color, but the cleared portion becomes a deep glaucous gray. Beneath the upper stratum a layer of light violet-gray appears, while a sediment of pale olive-buff collects at the bottom of the tubes. As the coagulum is slowly peptonized, the sediment and the deep glaucous-gray layer increase in depth; and when the coagulum has entirely disappeared the litmus becomes rapidly reduced. At the end of 25 days the tubes have lost all the litmus color, and are similar in color to peptone-beef bouillon. The blue has sometimes been restored by shaking; but at no stage was there any formation of acid.

**USCHINSKY'S SOLUTION.**—Growth in Uschinsky's solution is heavy, slightly turbid, with no surface growth; on the fourth day the medium becomes viridine-green. In old cultures there is a very slight sediment, which is slightly viscid.

**FERMI'S SOLUTION.**—Growth is scanty and uniform, with no surface growth; there is a slight green color similar to that produced in Uschinsky's solution.

**COHN'S SOLUTION.**—Growth in this medium is scanty, forming only a thin, uniform, scarcely visible clouding.

**STARCH JELLY.**—Starch jelly was made by adding 1 gm. of potato starch to 10 c. c. of Uschinsky's solution. Growth was moderate; the medium was turned to a fluorescent-green color. On adding an iodine solution to the medium a reddish-brown color was first formed, which on standing several minutes entirely disappeared, indicating the formation of achroodextrin, or even lower reduction products. There is a positive diastasic action.

**TOLERATION OF SODIUM CHLORID.**—Tubes of peptone-beef bouillon acidified to +10 and containing 1.5, 2.5, 3, 4, and 5 per cent of pure sodium chlorid were inoculated from 2-day-old agar slant cultures. Growth was moderate in the 1.5 and 2.5 per cent sodium-chlorid cultures, and was scanty in the 3 per cent cultures. In the cultures containing 4 per cent of sodium chlorid a clouding was formed, scarcely apparent to the eye, while in the 5 per cent cultures growth was entirely inhibited.

**BOUILLON OVER CHLOROFORM.**—Growth is unrestrained in +10 peptone-beef bouillon tubes to which 2 c. c. of chloroform have been added.

**FERMENTATION TUBES.**—The tests for gas and acid production were made in fermentation tubes containing neutral peptone-beef-litmus bouillon to which was added 2 per cent of the compound to be tested; these were dextrose, saccharose, maltose, mannite, and glycerin. Growth occurred most abundantly in the tubes containing dextrose and saccharose, but growth was abundant in all the sugar media. A reduction of the litmus took place in all the media, especially rapid in the glucose bouillon, the liquid finally becoming the color of standard peptone-beef bouillon. Neither gas nor acid was formed in the presence of any of the sugars after 15 days. In the presence of dextrose, saccharose, and maltose, clouding could be observed in the closed arms of the fermentation tubes; but in those tubes containing the bouillon with glycerin, mannite, and lactose, growth took place only in the open chambers.

**AMMONIA PRODUCTION.**—Peptone-beef-bouillon cultures tested at the end of the tenth day, using Nessler's reagent, gave a strong reaction for the presence of ammonia.

**REDUCTION OF NITRATES.**—Nitrates are not reduced. Five-day-old cultures tested both by the starch potassium-iodid test and by the sulphanilic-acid alphanaphthyl-amin test gave no color reaction.

**PRODUCTION OF INDOL.**—Tests made at the end of the tenth day on cultures in Dunham's peptone solution gave a strong indol reaction. The cultures tested with the sodium-nitrite sulphuric-acid reaction gave a strong pink color very quickly, while control tubes treated similarly remained uncolored.

**TOLERATION OF ACIDS.**—Neutral peptone-beef bouillon, to which were added 0.1, 0.2, 0.3, and 0.4 per cent, respectively, of citric, oxalic, and tartaric acids, was inoculated. After five days a moderate growth was visible in the cultures containing 0.1 per cent of all the acids used. This growth increased and was quite strong at the end of the fifteenth day, but in none of the 0.2, 0.3, or 0.4 per cent strengths of any of the acids was clouding visible, although kept for 30 days.

**TOLERATION OF SODIUM HYDROXID.**—The organism is quite sensitive to alkali. Peptone-beef bouillon titrated according to Fuller's scale to the following strengths: +35, +30, +20, +15, +10, +5, neutral, -5, -10, -20, and -30, were inoculated from a 24-hour-old +10 bouillon culture. Growth was apparent and vigorous in the +10 and +15 bouillon on the second day, and was also apparent, although scanty in those titrated to neutral, +5, and +20. On the third day tubes of -5 bouillon became slightly clouded and on the eighth day -10 bouillon and +30 bouillon showed a very scanty, scarcely visible clouding. Clouding in the -20 bouillon was not visible until after the tenth day, but on the twentieth day was easily apparent. Growth was never visible in the bouillon titrated to +35 and -30. The best growth took place in the +15 bouillon.

**HYDROGEN SULPHID.**—Strips of filter paper soaked in a saturated solution of lead acetate were suspended over cultures of the organism in a +10 peptone-beef bouillon. No browning of the paper was visible during five weeks' exposure. A medium of +10 nutrient bouillon containing 0.1 per cent of lead-acetate crystals was also inoculated with the organism. Although growth took place moderately, no precipitate of lead sulphid occurred in five weeks.

**METHYLENE-BLUE IN MILK.**—There is a very slow reduction of methylene-blue in milk. The organism was inoculated into tubes containing a milk medium to which 4 per cent of a 1 per cent solution of methylene-blue had been added. Slight reduction was visible on the third day, and apparently took place but slightly and very slowly. At the end of 15 to 20 days the original color of the milk had entirely disappeared; the coagulum was dissolved and the whey was a pale cendre-green, with an upper layer of dark green. This color did not disappear, although the cultures were kept for five weeks.

**SACCHAROSE BOUILLON.**—A medium of +10 peptone-beef bouillon containing 5 per cent of saccharose was inoculated. Growth was very vigorous, flocculent, and later formed a flaky, somewhat viscid sediment. In this medium filamentous chains of the organism were found.

**AEROBISM.**—The organism is aerobic in general; but is facultative anaerobic in the presence of dextrose, saccharose, or maltose. Stab cultures in tubes of +15 gelatin from which the oxygen had been removed by Wright's method<sup>1</sup> showed no growth. Likewise poured plates of +15 gelatin showed no growth underneath sterile cover glasses to exclude the air (Koch's method). However, in the closed arms of fermentation tubes containing glucose, saccharose, or maltose bouillon there is a distinctly visible growth.

**GROUP NUMBER.**—Following the numerical system of the Society of American Bacteriologists, the group number is 221.3332113.

<sup>1</sup> Mallory F. B., and Wright, J. H. Op. cit.

## EFFECT OF PHYSICAL CONDITIONS

## SENSITIVENESS TO DESICCATION

Drops of 24-hour cultures in +15 peptone-beef bouillon were placed upon sterile cover glasses in sterile petri dishes and kept in a dry dark-room. The organism was not killed after 12, 24, or 36 hours; but no growth was obtained after seven days of drying. On the other hand, although susceptible to drying in the air, old Citrus-blast material which had been kept for four months in the laboratory was found to yield the pathogenic organism. Under this condition, then, the organism is apparently able at least to live over the dry summer months.

## SENSITIVENESS TO SUNLIGHT

The Citrus-blast organism is rather sensitive to direct sunlight. Thinly sown agar plates from 10-day-old cultures in Uschinsky's solution and from 24-hour bouillon cultures were exposed bottom up to sunlight in September for 5, 10, 15, 30, 45, and 60 minutes. One-half of each plate was protected from the light by black paper, and in each case the plates were protected from heat by being placed upon ice.

Plates exposed for 30, 45, and 60 minutes showed no growth upon the parts exposed to the sunlight. Plates exposed for 15 minutes showed that 82 per cent were killed by the exposure, and those which survived grew more slowly than those unexposed. Plates exposed for 5 and 10 minutes showed colonies growing uniformly on the exposed as well as the unexposed sides.

## TEMPERATURE RELATIONS

The best growth obtained was at temperatures between 25° and 28° C. Scanty growth was observed at 16° and at 37° C.

The thermal death point determined by exposing newly inoculated peptone beef-bouillon cultures for 10 minutes to definite temperatures was found to be 50° C. In this case transfers were made from a 24-hour-old culture in peptone-beef bouillon to the bouillon tubes to be tested.

## ECONOMIC IMPORTANCE OF CITRUS BLAST

The chief injury caused by Citrus blast is in the killing of the young wood which should bear the blossoms and later the crop. Damage also results from defoliation and a consequent loss of leaf area for the whole tree. The whole result is a decrease in the ability of the tree to produce crops. The disease has also been observed in nurseries, and, although there is no means of knowing the actual loss suffered by this industry, the idea of dissemination by nursery stock immediately suggests itself.

As yet the disease has not caused any widespread damage, but the possibility of its becoming distributed throughout the State and perhaps other Citrus-growing regions is not to be disregarded.

## SUMMARY

(1) A new disease of Citrus trees is endemic to the Citrus regions of northern and central California. It has been described and given the name "Citrus blast" by Dr. J. E. Coit, of the University of California.

(2) Sections of fresh disease material show a bacterial organism present in masses, and on isolation plates bacterial colonies are obtained which on inoculation produce the typical lesions of the disease. From such positive inoculations the organism has been reisolated and reinoculated, giving positive results again, and finally being reisolated. The organism is apparently a new species, and the name "*Bacterium citrarefaciens*" is proposed.

(3) The organism exists in the parenchyma and destroys cell structure, leaving large pockets filled with bacterial masses. The organism does not ordinarily invade the vascular bundles and is apparently restricted to the parenchyma.

(4) The disease causes a decrease in leaf surface and a loss of the fruit-bearing wood in orchard trees. Young trees in nurseries may also be injured. The possibility of the disease becoming distributed throughout all California and other Citrus-growing regions is to be considered.



**PLATE A**

- 1, 2.—Orange twigs, showing brown blister-like scabs of Citrus blast, formed in summer over affected parts.
- 3.—Natural infection upon young leaves of a navel-orange tree.

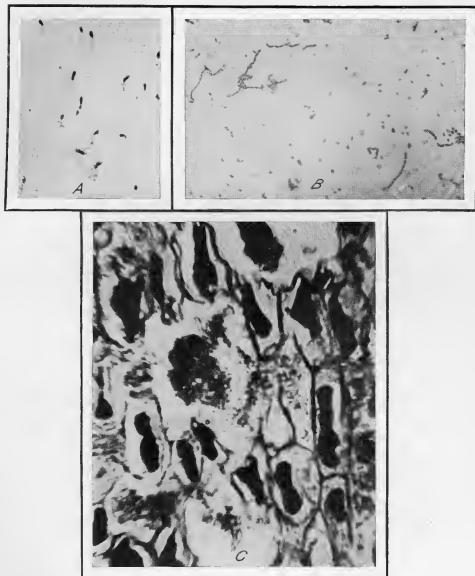


PLATE 1

A.—Flagella of *Bacterium citrarefaciens* from 24-hour-old agar slant. Stained by Löffler's method.  $\times 1,000$ .

B.—Filamentous bodies showing thickened areas of denser protoplasm from saccharose bouillon cultures of *Bact. citrarefaciens*. Stained with carbol-fuchsin.  $\times 450$ .

C.—Cross section of a navel-orange leaf affected with Citrus blast. Stained by Mallory's ferric-chlorid hematoxylin method. A few individuals and several masses of *Bact. citrarefaciens* may be observed.





**PLATE 2**

**Tree affected with Citrus blast, showing bared twigs and wilted leaves.**

## SOME FACTS ABOUT ABORTION DISEASE

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### CHARACTERISTICS OF THE ABORTION BACILLUS

While most bacterial diseases have two prime or main factors, a pathogenic microparasite and a susceptible host, infectious abortion disease of cattle is more complex, in that it has three prime factors, a pathogenic microparasite and two hosts. How imperfect our knowledge about this perplexing evil has remained at once becomes apparent when we consider that it has not been certainly determined which of the two hosts, the cow or the fetus, is primarily attacked by the microparasite. That is to say, we do not know whether the abortion bacillus primarily causes a disease of the cow's uterus which leads to the expulsion of the fetus, or whether, in the first place, it causes a disease of the fetus which subsequently impels the uterus to expel its contents.

One of the superlatively important facts about abortion disease is that cows often remain carriers of abortion bacilli long after they have ceased to abort, and that cows which have never aborted and regularly and normally produce seemingly healthy calves may be chronic carriers and disseminators of abortion bacilli.

As far as the writers have been able to learn, the abortion bacillus is an obligatory parasite. It may live and retain its virulence for a long time in infected material expelled from the uteri of infected cows, as such period of time can be measured through bacteriological cultivation and guinea-pig inoculation tests; but no data are available to support the belief that it can maintain itself or multiply under natural conditions as a saprophyte. Hence, the chronic persistence of the microparasite in the bodies of infected cows probably is the most important among the causes responsible for the propagation, the perpetuation, and wide prevalence of the disease.

The favorite habitat of the abortion bacillus in the bodies of cows is the udder, and the udder is seemingly its only habitat in the bodies of nonpregnant cows. Our work regarding this fact includes hundreds of carefully made tests with milk from numerous cows. Some of the cows had aborted, and others had not; the milk of some was infected with abortion bacilli continuously, and that of others intermittently; that of some cows remained infected year after year and that of others for shorter periods of time. In one case (a cow that remained under observation for seven years) periodic tests proved the milk to be infected continuously.

Another fact related to the expulsion of abortion bacilli with milk from the udders of cows is that in the numerous tests made with milk from many different cows the abortion bacillus was never found in the milk of a cow unless both her milk and her blood serum possessed agglutinating properties for suspensions of abortion bacilli. This fact is interesting and important not only on its own account but also because it serves as strong circumstantial evidence to prove that the work of the writers on the occurrence of abortion bacilli in the milk of cows is trustworthy. It does not mean, however, that the milk of all cows which react with the agglutination test for abortion disease is infected, as the writers have repeatedly tested milk from reacting cows without detecting abortion bacilli.

Regarding reacting cows with uninfected udders, it appears that their blood serum gradually loses its power to agglutinate suspensions of abortion bacilli. The writers wish, however, to have this statement taken cautiously, as the evidence behind it is not yet sufficient to give it the rank of a proved fact. If this statement, on further study, should prove true, it, together with other facts, will justify the conclusion that the persistence of agglutinating and complement-fixing substances in the blood of cows, relative to abortion disease, is intimately associated with the abortion bacilli that enter the body through the lymphatics from infected udders, as abortion bacilli do not maintain themselves in the bodies of cows elsewhere than their udders and gravid uteri.

That abortion bacilli do not maintain themselves in the bodies of cows elsewhere than the regions named is a fact of which the writers have obtained fairly convincing proof. It was found that abortion bacilli injected into the veins of normal, nonpregnant cows disappeared from their circulating blood in the course of a few hours; and when such cows were killed sometime afterwards, though their blood had become positive with agglutination tests, the germs could not be found in their bodies unless it was in their udders and associated lymph glands. One case in the records of experiments is remarkably impressive as an illustration of the tendency of abortion bacilli to lodge in the udder. The case is that of an adult, virgin, female animal, a heifer, approximately 4 years old, which was given an injection of abortion bacilli into one of her jugular veins. Later it was found that the infection<sup>1</sup> had established itself in her virgin udder, which was not functioning and never had functioned.

Another series of tests, probably even more convincing than the foregoing, was a careful search for abortion bacilli in the bodies of naturally infected as distinct from artificially infected cows. The cows were killed and their blood, spleens, livers, kidneys, brains, ovaries, uteri, udders, milk, synovial fluid from various joints, nerve tissue, lymph glands from all portions of the body, etc. tested for abortion bacilli

<sup>1</sup> The term "infection" is used here and elsewhere in this paper as signifying the discoverable presence of abortion bacilli, and not as implying the development of observable lesions of disease.



through animal inoculation and cultural methods, with the following results: In all cases two or more quarters of the udder, the milk from the infected quarters, and one or more supramammary lymph glands, and in one instance some of the pelvic lymph glands, were infected. All other organs and tissues were invariably free from infection.

When abortion bacilli are injected into the nonpregnant uterus of a cow, they disappear in the course of a few days. When the discharge from the uterus of a cow which has aborted is tested, abortion bacilli for 20, 30, or even 40 or 50 days may be found; but they eventually disappear, and it is the impression of the writers that their abundance and period of persistence are intimately related to the magnitude of the lesions in the uterus attendant upon an abortion.

It is the belief of the writers that the evidence they have supplied is sufficient to prove two facts: (1) that the udders of cows are a common habitat of abortion bacilli, and (2) that abortion bacilli do not maintain themselves in the bodies of nonpregnant cows elsewhere than in their udders. The occurrence of the bacilli in the supramammary glands, and in one instance in pelvic lymph glands, and no farther in the body, merely proves that the germs tend to penetrate into the body from the udder through the lymph channels, but that they can not go very far before they are destroyed.

#### PRODUCTION OF SEEMINGLY NORMAL CALVES BY INFECTED COWS

When abortion bacilli are injected into the udder through the teat, by a method which avoids a trauma, the bacilli are established in the udder, and the cow, according to all available tests, becomes an infected cow.

There is a remarkable and truly important fact concerning the production of calves by cows with infected udders. Such cows, irrespective of whether they have, at some time in the past, aborted or not, may give birth to seemingly normal calves in a seemingly normal manner associated with the occurrence of abortion bacilli in their uteri and in the afterbirth. Quite a number of records prove this, and although it does not occur every time a cow with an infected udder calves, it is far from uncommon. As has been stated, it may occur with a cow which has never aborted; and it may occur with the third seemingly normal parturition after an abortion. In the experience of the writers, in which they have made a number of tests, this remarkable fact has never been observed in connection with cows which react positively with the agglutination test but the udders of which were free from infection. And the fact becomes all the more remarkable when it is viewed in the light of another fact—namely, that numerous careful tests of the uteri of nonpregnant cows, irrespective of whether their udders were infected or not, tests made both between and during periods of œstrum, in no instance revealed the presence of abortion bacilli.

Another fact which merits consideration in this connection was derived from tests with newly born calves. A number of calves produced by cows with infected udders were killed immediately after they were born and their bodies tested for the presence of abortion bacilli through guinea-pig inoculation methods. These calves were not permitted to come into contact with their mothers or other sources of infection that would tend to introduce germs into their bodies not present at the moment of completed parturition. It was found that such calves—those that were delivered alive and seemingly vigorous and healthy—may harbor abortion bacilli in their stomachs and gastrohepatic lymph glands; but invariably, when the calves were infected, the afterbirth and the uteri of their dams were also infected. In aborted fetuses the stomachs, intestines, lymph glands, spleens, livers, blood, and subcutaneous extravasations of serum may contain abortion bacilli.

#### EXPERIMENTAL INFECTION INTRODUCED THROUGH TEAT

One record of the injection of abortion bacilli into the udder of a cow, through the teat without trauma, is particularly interesting. The cow was well advanced in pregnancy and, according to all tests that could be made, was free from abortion disease prior to the injection. This record is given in detail because it is very instructive and also illustrates the laborious application the investigation of abortion disease requires. In this connection it may be observed that in this work the writers have used the agglutination test rather than the complement-fixation test for abortion disease. The reason for this is that the writers are convinced that the agglutination test for this disease is fully as reliable as the complement-fixation test, but far less complex; hence, in the hands of those who have many and varied duties, it is more reliable.

#### RECORD OF COW 1154

September 9, 1914. Received at the experiment station from an abortion-free herd. About 8 years old. Was negative to all tests for abortion disease and was carefully protected against exposure to infection.

August 21, 1915. Served by bull 1150 and conceived. The bull was received at the station on the same day on which the cow was received, and was and is now negative to all tests for abortion disease, and has been carefully protected against exposure to infection.

December 10, 1915. Agglutination tests with blood serum from the cow and the bull were made. Negative in both cases.

March 27, 1916. Agglutination tests with blood serum from the cow and the bull were made. Negative in both cases.

March 27, 28, 29, 30, 31, 1916. Material was obtained on each day from the udder of the cow and injected into guinea pigs. The guinea pigs were subsequently killed and examined post mortem and found to be free from lesions of the kind caused in guinea pigs by abortion bacilli; in fact, they had remained perfectly healthy and showed no lesions of any kind.

April 3, 1916. The growth on two culture tubes of abortion bacilli was scraped off and suspended in 30 c. c. of sterile normal salt solution and injected into

the right front teat of the cow. The method of injection was through gravity, and the pressure used did not exceed that exerted by a column of fluid 12 inches high. Two guinea pigs were injected with samples of the suspension, and both later showed typical lesions of the kind caused in guinea pigs by abortion bacilli.

April 8, 1916. Five days after the injection agglutination tests with blood serum from the cow were negative.

April 17, 1916. Two weeks after the injection agglutination tests with blood serum from the cow were positive with dilutions of 1 to 400, which must be regarded as a very strong reaction.

April 22, 1916. Material from the infected quarter of the cow's udder was injected into guinea pigs, which subsequently developed typical abortion-bacillus lesions.

May 3, 1916. Material from each quarter of the cow's udder was injected separately into guinea pigs, all of which subsequently developed typical abortion-bacillus lesions, showing that the infection originally introduced into one quarter had spread to the other three quarters. On the same day material from the udder agglutinated suspensions of abortion bacilli in the following dilutions:

Right front, or injected quarter.....	1 to 6,400
Left front quarter.....	1 to 1,600
Right hind quarter.....	1 to 800
Left hind quarter.....	1 to 1,600

It is interesting to note how much higher the agglutinating value of material from the injected quarter is than from the other quarters. The material obtained from the cow's udder is not called "milk," because the cow was practically dry; and it is questionable whether the material which can be stripped from a practically dry udder shortly before parturition can reasonably be looked upon as milk.

May 9, 1916. The agglutinating value of material from the injected quarter of the udder was positive in a dilution of 1 to 12,800, and on May 15, 19, and 24, in a dilution of 1 to 25,600. On these days the agglutinating value for suspensions of abortion bacilli of material from the other quarters of the udder remained constant for a dilution of 1 to 1,600, and that of the blood serum of the cow for a dilution of 1 to 400.

May 26, 1916 (279 days after service by the bull). The cow produced an undersized, weak calf, which, however, rapidly gained strength and is now a normal, healthy, vigorous animal. On the day of parturition the following agglutination tests were made:

Colostrum, injected quarter of udder, positive, dilution 1 to 25,600.
Colostrum, other three-quarters of udder, positive, dilution 1 to 1,600.
Blood serum, cow, positive, dilution 1 to 400.
Blood serum, calf, positive, dilution 1 to 400.

When agglutination tests are made with blood serum, it is common for newly born calves of infected cows to react in the same dilutions or quite as strongly as their mothers, but this power to react does not persist; it is a rapidly declining phenomenon, as is well shown by the following tests of the blood serum of the calf concerned in this record.

On the day of its birth, as above recorded, the agglutination value of the calf's blood serum and that of its mother were identical; positive in dilutions of 1 to 400. Seven days later, June 2, the agglutination value of the calf's blood had declined to 1 to 200; on June 7 it had fallen to 1 to 100; on June 9 it was still at 1 to 100; but on July 10

all agglutinating power for suspensions of abortion bacilli had disappeared.

Contrary to this, the agglutinating power of the cow's blood serum remained constant for dilutions of 1 to 400. Not so, however, with the agglutinating power of material from her udder. Colostrum, as has been seen, agglutinated in dilutions, injected quarter, 1 to 25,600; other quarters 1 to 1,600. The milk as early as June 8, or 13 days after parturition, was positive in dilutions no higher than 1 to 200 in the injected quarter, and 1 to 50 in the other quarters, at which points it remained fairly constant.

The most interesting fact about this cow was that parturition was associated with retention of the afterbirth, which, on removal, was found to contain much abnormal material of a yellowish color, and this was proved to be infected with abortion bacilli. Vaginal discharge from the cow was also proved to be infected with abortion bacilli on June 1, 3, and 12, and free from infection on and after June 20.

#### THE UDDER AS A POSITIVE CHANNEL OF INFECTION

This one cow illustrates a number of abortion-disease phenomena. First, the introduction of abortion bacilli into the udder through the teat, though a method of injection was used which almost certainly precluded mechanical injury, positively infected it and caused the gradual development of agglutinating power for suspensions of abortion bacilli in the blood serum. In other words, the udder is a possible channel through which abortion bacilli may penetrate into the body.

Second, the passage of abortion bacilli from the udder to the uterus is an experimentally demonstrated fact. The writers have already stated that, in all cases in which they found abortion bacilli in the uterus after seemingly normal parturitions, the cows had infected udders; and it is only necessary to add that, in practically half of the cows with infected udders that have been examined relative to this matter, the uterus and placenta were infected with abortion bacilli.

It has been suggested that the abortion disease may perpetuate itself through abortion bacilli that enter the udder through the teat. When we consider how cows are milked, and how the milker goes from cow to cow without disinfecting his hands, and that the udders of cows are the commonest habitat of abortion bacilli, this mode of infection can not be regarded too lightly, or as an untenable supposition. That this is a means of perpetuation has not been proved, but it should be considered as a possibility.

In the third place, the record of cow 1154 illustrates another fact—namely, the high agglutinating power of colostrum from cows with infected udders. This phenomenon, together with the rapid decline of agglutinating power of material from the udder as milk takes the place of colostrum, has been repeatedly observed.



In the fourth place, the rapidly declining agglutinating power of the blood serum of the calf of an infected cow is shown, and this also is a repeatedly observed phenomenon. The writers have found that agglutinating properties can be engendered in the blood of calves by injecting them with abortion bacilli; but such injections must be repeated from time to time, otherwise the agglutinating properties of the blood serum disappear.

In the fifth place, as the calf was suckled by its mother, whose udder was known to be heavily infected, it may be judged from the rapidly declining agglutinating value of its blood that abortion bacilli in ingested milk do not seem to penetrate deeply or abundantly into a calf's body. The records of other cows and calves give similar data.

#### POSSIBILITY OF INFECTION THROUGH THE BULL

It is rare for male and virgin cattle to react positively to abortion tests, and it has been pointed out that the bodies of cows do not harbor abortion bacilli elsewhere than in their udders, associated lymph glands, and pregnant uteri. It does happen occasionally that bulls do react when they are tested for abortion disease, and what such reactions may signify remains decidedly questionable; hence, the two following cases may be both instructive and interesting.

Sometime ago the writers found two bulls which reacted when their blood serum was tested with suspensions of abortion bacilli. In one case the reaction was positive in a dilution of 1 to 200 and in the other in a dilution of 1 to 100. Where the bulls got the infection the knowledge of their history does not reveal.

One of the bulls, the one with the higher reaction, was immediately killed and examined. The only lesion found in his body was an abscess involving the epididymis of one testicle, and this abscess was definitely proved to be infected with abortion bacilli. Tests of all other portions of the sexual organs and various other organs of the body failed to reveal abortion bacilli.

Was this apparently healthy bull qualified to serve as an active disseminator of abortion disease? The writers are not ready to answer the question at present.

The other bull was permitted to serve a cow which, according to her history and all tests made, was free from abortion disease. Immediately after the service seminal fluid was recovered from her uterus and injected into a number of guinea pigs, one of which subsequently showed abortion bacillus lesions. Tests are still being carried on with this bull.

#### RELATION OF THE ABORTION BACILLUS TO THE EMBRYO OR FETUS

A few years ago one of the writers, on the basis of the work on abortion disease, expressed the view that the abortion bacillus seemed to have a peculiar affinity for embryonic tissue. They are still of this opinion, and

it is possible that the disease is in fact primarily a disease of the embryo or fetus rather than of its mother. The mother, to be sure, is the source of infection. Possibly, however, if a large enough number of virulent abortion bacilli are poured into her body from her udder, antibodies of sufficient potency may develop in her blood to protect her fetus. Should this prove true, good results in the treatment of infected herds may be expected from injections into the mother, possibly a short time before she conceives or early during pregnancy, of cultures of abortion bacilli; and it is possible in this case that the more virulent the cultures are and the more abundant the material injected the better the results will be.

#### CONCLUSION

To prevent the further spread of abortion disease, owners of uninfected cattle should be instructed to have careful agglutination tests for abortion disease made of all cattle they propose to introduce into their herds; and owners of infected herds should be taught that aborted fetuses, also the afterbirth and discharge from the vaginas of infected cows, are infected with abortion bacilli and must therefore be disposed of with care.

The treatment of individual cows which have aborted or failed to clean properly after parturition must be left largely to the good judgment of the practicing veterinarian. If the uterus is given a proper chance to heal after it has been damaged by an abortion or a retained afterbirth, the abortion bacilli in it need occasion little worry, as they will rapidly disappear of their own accord, and it is very questionable whether reparative processes are not retarded rather than facilitated by douching with germicidal solutions which are strong enough to kill bacteria in a reasonable length of time, or the length of time during which they may remain undiluted in the uterus. Douching is no doubt good practice, but it is desirable that there be a flooding out, a washing out, a real physical cleaning of the uterus; and this can best be done with solutions which are healing rather than germicidal, soothing and not irritating.

# WHEAT-SHEATH MINER

By H. L. SEAMANS,

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## INTRODUCTION

In an investigation in 1915 of wheat plants (*Triticum* spp.) supposed to be infested with *Meromyza americana* Fitch, two different forms of larvæ were noticed, one a greenish larva, apparently that of *M. americana*, and the other a smaller, whitish one. Further examination showed the latter to be more plentiful and that the damage to wheat plants supposed to be caused by the *M. americana* was mainly due to the other species. A few plants were taken to the insectary at Bozeman, Mont., and the insects on coming to maturity were found to be mainly *Cerodonta femoralis* (Meigen), a species about which very little has previously been known.

## HISTORY OF THE SPECIES

This insect was first described by Meigen in 1838 (2, p. 397)<sup>1</sup> as *Agromyza femoralis*. At that time the genus *Agromyza* Fallen embraced a number of species now placed in genera since split off. In 1835, however, Macquart (1, p. 214) had used the name "Odontocera" to designate the genus which in 1861 Rondani (3, p. 10) called "Cerodonta;" but, "Odontocera" being preoccupied, it can not be used here. In 1862 Schiner (4 and 5) gave this genus the name "Ceratomyza," and this is the name now used by some authors. Later writers on this group of flies, Melander (7) and Malloch (8, p. 331), have used Rondani's "Cerodonta" for this genus, which has priority over "Ceratomyza"; therefore Meigen's *Agromyza femoralis* then becomes *Cerodonta femoralis*. A search of the literature reveals practically nothing about the biology of this insect. Neither Aldrich nor Malloch discusses it, although Aldrich includes a note under the genus *Ceratomyza*, which reads:

An undescribed *Ceratomyza* has been reared from young wheat plants at Pullman, Wash., by Prof. C. V. Piper; it causes considerable damage.

According to recent information from Melander, who is now Entomologist at the Washington Agricultural Experiment Station, this fly was *C. femoralis*. Melander (8) mentions the species only in a key to the genus *Cerodonta*.

## DISTRIBUTION OF THE INSECT

Melander (8) reports having specimens from Europe, Montana, Wyoming, Idaho, Washington, Oregon, California, and British Columbia. This would indicate that it is generally distributed in the Northwest.

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<sup>1</sup> Reference is made by number to "Literature cited," p. 24-25.

In Montana injury has been reported, and specimens have been received in material from the following towns: Arlee, Ronan, Twin Bridges, Brady, and Seventynine. This covers pretty well the whole western portion of the State, though perhaps the most severe damage has been done at Arlee. Most of the material for this work was collected there.

#### HOST PLANTS

Specimens of *C. femoralis* were found in or reared from winter wheat, spring wheat, oats (*Avena sativa*), and timothy (*Phleum pratense*). The latter was found growing abundantly along the ditches and fences surrounding the field. Volunteer wheat and oats were also found to be infested during the latter part of September. As this insect has been found only in plants belonging to the Graminae, it appears that native grasses may be its natural host plant.

#### DESCRIPTIONS OF THE LIFE STAGES

##### EGG

The egg is ellipsoidal, white, soft, smooth, translucent, glistening, 0.5 mm. long, and from 0.15 to 0.2 mm. broad.

##### LARVA

The larva is white tinged with green, excepting for the blackish or brownish alimentary canal, which shows through the body wall. It is about 3.0 mm. long and 0.5 mm. in diameter when full grown. There are 13 segments. The mouth parts are indistinct, but are represented by black, chitinous hooks. There are two tubercles on the median line at the anterior end on the ventral surface, one each on the second and third segments. The first three and the last segments are short, the rest being of equal length, are slightly wider than long. The oral opening is found on the ventral side of the first segment and the anal opening on the ventral side of the last segment.

There are two pairs of respiratory organs, or breathing appendages, one at each end of the body. They are similar in structure, and those on the same side are connected by a tracheal tube, which is visible through the larval skin as a fine, white thread. Each appendage consists of a main stalk which branches into five smaller stalks, at almost right angles. Each of these small stalks has from one to three openings, the total number varying from 8 to 11.

The anterior segment of the body bears a patch of short black spines, placed just above the mouth hooks.

##### PUPARIUM AND PUPA

The puparium is moderately chitinized, brownish, semitransparent, 3.0 mm. long and 1.25 mm. broad in the middle, slightly tapering toward the ends and somewhat flattened dorsoventrally. There are 10 plainly visible segments, the anterior and posterior ones being hemispherical.



Toward the end of the pupal period the pupa is visible through the puparium, showing the parts of the body distinctly, and movements in the legs can be seen three days before the emergence of the adult fly.

#### ADULT

A translation of Meigen's description (2, p. 398) of the adult fly is as follows:

Black; head, pleuræ, and femora yellow; third antennal joint black, with distal thorn. Head light yellow, with black ocellar spot. Base of antennæ yellow, third joint deep black, with black arista and apical spine. Notum and scutellum shining black, pleuræ yellow, abdomen black and shining. Femora yellow, tibiæ and tarsi piceous. Halteres white; wings grayish.  $\frac{2}{3}$  line.

From an abundance of material of both sexes the following description has been prepared:

#### *Cerodonta femoralis* (Meigen).

Adult male: Length, 2.0 to 2.5 mm.; wing expanse, 4.0 mm.

Head as broad as the thorax; front broad, about one-third the width of the head, yellow except for a rectangular, black, ocellar spot at the vertex. Three pairs of frontal bristles present, reaching to the base of the antennæ; one pair of ocellar bristles directed forward, situated at the two anterior corners of the ocellar spot; two pairs of vertical cephalic bristles, the outer pair divergent, the inner pair convergent; one pair divergent post vertical bristles present, located at the posterior margin of the ocellar spot. The three ocelli are located, one in the middle of the anterior margin, and the others in the middle of each of the lateral margins of the ocellar spot; ptilinum visible as a raised triangular portion, just above the base of the antennæ. Oral vibrissæ present, with four pairs of small hairs along the oral margin. Oral region, including the labella and genæ, bright yellow.

Antennæ three-jointed, the first joint small, ringlike, and yellow; second joint larger, brown in color, with one medium-sized bristle, and a coronet of small bristles; third joint the largest, black oval, with a dorsal arista and an apical spur, pubescent.

Occiput black; postorbital bristles present.

Thorax for the most part shining black; pleural sclerites yellowish, or gray bordered with yellow. Four pairs of dorso central, one pair each of humeral, posthumeral, notopleural, supraalar, postalar, and scutellar apical bristles present. One propleural bristle, and one large and from two to four small mesopleural bristles present; sternopleurite with one bristle below the sternopleural suture, and a fanlike row of bristles above the middle coxa.

Anterior coxæ large, femora bowed; all coxæ with bristles; femora with a small bristle on the posterior surface; anterior tibia alone without apical spine; coxæ and femora yellow, tibia and tarsi brown.

Wings grayish, basal portions of veins yellow, darker toward the tip; halteres whitish or yellow; squamæ small, colorless.

Abdomen viewed dorsally with six easily distinguished segments (the sixth is a genital segment) tapering posteriorly, uniformly hairy, black, except for a narrow yellowish line on the posterior margin of the first two and sometimes all of the segments. The fifth segment is about twice as long as the preceding; the sixth is somewhat globular in shape, with the posterior and ventral surfaces cleft, so that the two lateral plates form a pair of claspers. A chitinous process may protrude through the posterior cleft, and the penis hinges on this process, being directed forward. The dorsal surface of the penis near the hinge is open and the vas deferens, a flexible tube, follows along the chitinous process and enters the penis at the dorsal opening. The chitinous process upon which the penis is hinged appears to extend to the second

segment. When not protruded, the chitinous process is drawn up into the body, sheathing the penis within the ventral portion of the fifth segment.

The size of the female is the same as that of the male, and in general description the two sexes are alike. Abdomen less tapering, six segments, a seventh segment sometimes visible when the ovipositor is protruded. Ovipositor retractile, tubular, with a hard, chitinous edge. Posterior segment not cleft ventrally.

#### NATURE AND EXTENT OF INJURY

A field of wheat infested with the wheat-sheath miner may not appear to be greatly injured. Unless the field is badly infested the grain has a

healthy color and appears to be strong. A badly infested field appears slightly off color and may show areas which look decidedly unhealthy. It is necessary to make a close examination of individual stools to even begin to estimate the real damage. This is true especially of winter wheat, where many of the stems of the fall growth may be present only as dried and withered leaves.

The injured culms are instantly recognized by the fact that, while the leaves, or at least part of them, are green and apparently healthy, the central stalk is dead and withered (fig. 1). The injury done by *C. femoralis* appears identical

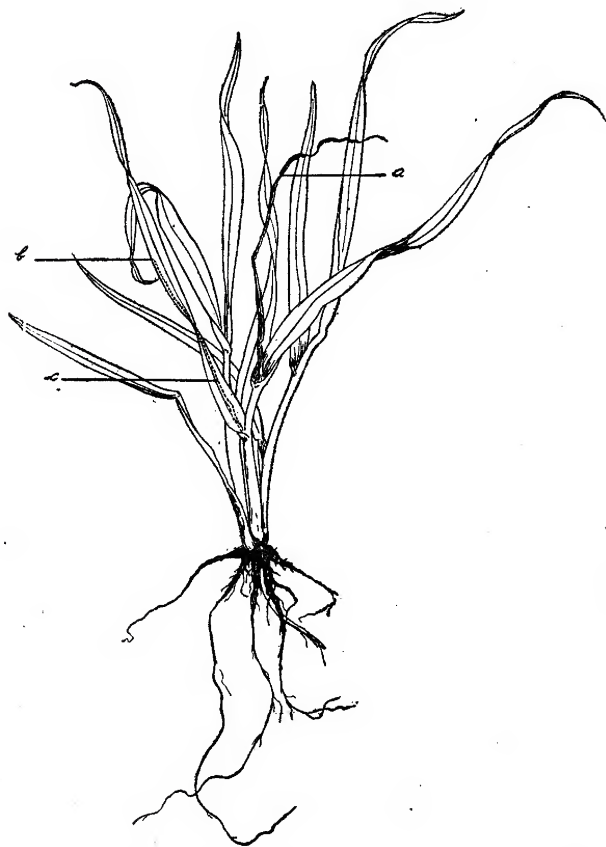


FIG. 1.—Young wheat plant, showing (a) central stalk injured by the wheat-sheath miner, *Cerodonta femoralis*; (b) point where egg was laid; (c) mine in the leaf made by larva on its way to the sheath; other leaves normal.

with that of *Meromyza americana* until the leaves are examined. In each case the larva enters the leaf sheath by mining down from the point in the leaf where the egg was laid. If the injury is due to *C. femoralis*, the mine in the leaf is narrow, clean-cut, and almost straight; while if it is done by *M. americana* it is broad and irregular with indistinct edges. While *M. americana* enters the stem and eats out the central stalk, usually cutting it off above the first node, *C. femoralis* confines its attack to

mining up and down the leaf sheath and sometimes girdling the stem without completely cutting it off. Whether it girdles the stem or not, the injuries caused by mining in the sheath appear to be sufficient to kill the stalk.

Some of the farmers who were interviewed in regard to the damage done by this insect estimated that their yields of winter wheat had been cut down at least 25 per cent in the season of 1915. Though new shoots often spring up from the lower nodes after the central stalk is killed, these are not as strong and do not bear as large or as full heads of grain as the normal stalk.

A field of spring wheat was visited in 1916, in which by actual count 95 per cent of the plants showed injury by this insect. Some of these plants had only one culm injured, while others had lost two or three. An adjoining field of oats was found to have 12 per cent of its plants infested.

There is a slight amount of injury done to the plants, just before blossoming, by the second brood of larvæ. This injury is only to the leaves and probably has little or no effect on the yield, as the central stalk does not seem to be injured.

To what extent the larvæ injure winter wheat in the fall has not yet been ascertained. Farmers have stated that they have seen the injury in winter wheat, but it has been impossible to make an investigation of this statement because very little winter wheat has been grown in the infested localities in the last two years.

#### LIFE HISTORY AND HABITS OF THE MINER

The study of the life history of the wheat-sheath miner was carried on in the insectary at Bozeman, with material collected in the vicinity of Arlee. Several plants containing eggs and larvæ were collected at Arlee on June 7, 1915, and were brought in for the purpose of rearing a supply of adults and securing all the data possible regarding this species.

Adults emerged in the insectary on July 11 and continued to emerge until July 24. These adults were kept alive for some time, and their habits were studied in various ways. The first flies to emerge were placed in lamp-chimney cages over seedling wheat plants. The females fed on the juices of the wheat plants by making tiny incisions in the upper surface of the leaves and then lapping up the juices which exuded. One female fly caged over a wheat plant made 102 feeding punctures in 24 hours. The males showed no inclination to feed on these juices; but when some wheat blossoms were placed in the cages they appeared to feed on the pollen, touching the anthers continually with the labella. The flies began feeding almost immediately on emerging, and one newly emerged female was seen to make three feeding punctures in five minutes.

Copulation may take place any time after 24 hours from emergence, and this is followed by a preoviposition period of about three days. The oviposition period in the insectary was about 10 days, but is, without doubt, longer under natural conditions.

The plants which were brought in from the field on June 7 yielded flies from July 11 to July 24. As there was no possibility of eggs being laid after June 7 in those particular plants, theoretically the last eggs laid produced flies that emerged on July 24. If all the flies completed their life cycle relatively in the same length of time, the eggs which produced the flies emerging on July 11 must have been laid 13 days earlier than June 7, or on May 25. As the flies were plentiful on June 7 and were still laying eggs, it is probable that the oviposition period lasts about three weeks, or from May 20 to June 10.

The number of eggs laid by a single female was not ascertained, though six flies, caged over wheat plants, laid 96 eggs in 24 hours, which gives an average of 16 eggs per fly for that time.

The egg-laying process is simple and takes from 7 to 12 seconds. The ovipositor is brought at right angles to the surface of the leaf, the upper epidermis is punctured, and the ovipositor is forced underneath it, toward the base of the leaf. A contraction of the abdomen forces the egg into the lower end of the puncture and the ovipositor is withdrawn, leaving the egg well protected under the epidermis. The feeding punctures are made in a similar manner, except that the leaf surface is scraped instead of the epidermis being punctured.

The incubation period of the eggs is about six days under insectary conditions. Eggs were laid in wheat plants on two consecutive days, fresh plants being substituted each day. These eggs were watched, and six days later the first ones hatched and were followed by the rest the next day.

Immediately on hatching from the egg the larva starts mining down the leaf toward the stalk, eventually ending in the leaf sheath, at the crown of the plant, or at the first node. On reaching the base of the leaf sheath the larva feeds up and down the sheath, and sometimes around the stalk.

The length of the larval period is variable, as some of the larvæ pupated at the end of 10 days, while some took as long as 20 days, depending on weather conditions. Cool, wet weather seemed to delay pupation.

Some of the larvæ were removed from the leaf sheath and allowed to pupate in a tin box. Others were left in the plants to pupate normally. They did not leave the sheath, but pupated either at the node or down close to the crown of the plant. None of them entered the soil or worked into the center of the stalk for pupation.

The pupal period lasts about 25 days under insectary conditions. Larvæ pupated in plants and in tin boxes on June 22 and emerged on July 16 and 17. What the variation in the length of the pupal period would be under varying weather conditions can not be estimated. As an indication that insectary conditions were parallel with natural conditions, wheat plants that were sent in from the field on the same date contained pupæ from which flies were emerging.



## SEASONAL HISTORY

There is some doubt as to the number of broods of the wheat-sheath miner in a year, though there seem to be three full broods, with the hibernation spent in the pupal stage.

The adult flies appear about May 20 and lay eggs in wheat seedlings until about June 10. On June 16, 1916, no flies were caught over a badly infested field of wheat, though the day was ideal for making a collection. The flies were apparently through ovipositing and had disappeared by that time.

The flies of the second brood emerged in the insectary about July 16, and lived until August 5. As pupæ were sent in from the field about that time, from which flies were emerging, it appears that the second brood is present in the fields from about July 15 to August 10. This brood lays eggs in the leaves of the wheat and also in seedling volunteer wheat and grasses. The life cycle of this brood was carried out in the insectary, and the third generation of flies began to emerge September 7. By September 10 all the pupæ in the cages had emerged and the flies were put out of doors with wheat plants, to keep them under more normal conditions. They did not survive a sudden cold snap which occurred at Bozeman on September 13.

The next generation, which is believed to hibernate as pupæ and produce the first brood of flies the following spring, has never been reared in the insectary; and it is only by means of a few scattering facts that the occurrence of this generation is suspected.

Many farmers who were questioned concerning this insect told the writer that they had often found "a small, brown, wrinkled egg down next to the killed stalk in the winter wheat during the fall and winter." By this they doubtless meant that they had noticed the pupa in the dead stalk. Some of these men had noticed the same thing in the stubble after cutting in August. Since winter wheat is planted after the middle of August and the second brood of flies is apparently through ovipositing before the wheat has come up, the pupæ in the winter wheat must have been the result of eggs laid by the third brood of flies. During March, samples of winter wheat were sent in which contained pupæ apparently identical with those of *C. femoralis*. These pupæ never emerged, and the identity of the flies could not be definitely determined, though there is little doubt of their being *C. femoralis*. During the last week in September, 1916, a visit was made to the badly infested locality. Pupæ and fully grown larvæ were found in timothy, volunteer oats, and wheat. These were apparently the third brood and indicate the pupal hibernation, thus assuring three broods for the year.

It does not seem likely that the pupæ of the second brood would last through the fall and winter, since all those in the insectary had emerged by September 10. Neither does it seem likely that the adults would over-

winter and lay eggs in the spring, since those which emerged were killed by a sudden cold snap. While the climate at Arlee is warmer than that at Bozeman, the winter there is considerably colder than a September cold snap at Bozeman and more liable to be injurious to insect life.

#### PARASITES OF THE MINER

Two hymenopterous parasites were reared from the puparia of *C. femoralis*. The puparia which are parasitized are of a darker color, with the segmentations more distinct than in normal puparia. These parasites were determined by Mr. A. B. Gahan, of the Bureau of Entomology, through the courtesy of Dr. L. O. Howard, to be a new species of *Dacnusa* (Braconidae) and *Cyrtogaster occidentalis* (Chalcididae). There were not enough of either of these species to be effective agents in control.

#### CONTROL

No control measures have as yet been tried, but a knowledge of the seasonal history of the fly leads to suggestions. At the time wheat is cut for harvest, the larvæ of the second brood are in about the last instar at the crown of the plant, or have already pupated. Scattering the straw over the field and burning the stubble as well as grass borders surrounding the fields would doubtless get rid of a high percentage of the flies. Should this insect ever become a very important factor in grain growing, it is possible that it would be desirable to use a header or cut the grain very high, either of which would leave the straw on the field where it could be burned. However, if burning the stubble is not practicable, plowing it under about 6 inches and harrowing just after removing the crop or before planting a spring crop would probably bury the pupæ deep enough to prevent the flies from emerging.

The late seeding of winter wheat, after a thorough destruction of volunteer grain and grass, would doubtless accomplish much in control. The wheat could be sown about the third week in September, and would not be up until after the greater number of flies had finished ovipositing. This would not only prevent the injury to the wheat itself, but would do away with the main source of infestation in the spring.

As native grasses are probably the natural hosts of the fly, crop rotation would be almost useless. However, the cleaning up of field borders and destruction of volunteer stools during the fallowing period would aid greatly in the control of this insect.

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## RELATION OF THE WATER-RETAINING CAPACITY OF A SOIL TO ITS HYGROSCOPIC COEFFICIENT<sup>1</sup>

By FREDERICK J. ALWAY, *Chief of Division of Soils*, and GUY R. McDOLLE, *Assistant in Soils, Agricultural Experiment Station, University of Minnesota*

### INTRODUCTION

In recent years the importance of the water contained in the deeper portions of the subsoil—that below the depth penetrated by the roots of crop plants—has been a much-discussed question, and most extreme views are entertained, both as to its rôle in the moisture supply of annual crop plants in dry-land regions and as to its influence upon the indefinite maintenance of the mineral nutrients in the surface soil.

In a recent analysis of the outlook for the reclamation of nonirrigable lands in regions of very low rainfall, Hall (12)<sup>2</sup>, while mentioning that some work in widely separated regions has cast doubt upon the common supposition that—

the subsoil below the actual range of the roots of the crop may still return water by capillarity to the higher levels that are being depleted, the deeper subsoil thus acting as a kind of regulating reservoir absorbing rain in times of excess and returning it when the need arises—

has pointed out that—

the evidence on either side is far from being conclusive and more experiment is very desirable (12, p. 641).

The present differences in views appear to be due to the failure in laboratory experiments and field studies to take into consideration some physical constant that is directly related to both the lower limit of available moisture and the water-retaining capacity of the soil, if we define the latter as the maximum amount which a soil will carry after it has been saturated and then, protected from both direct evaporation and the indirect effects of this as well as the action of plant roots, allowed to come into approximate moisture equilibrium by the downward movement of the excess of water into the subsoil mass. The lower limit of available moisture as

<sup>1</sup> The work reported in this paper was carried out in 1912-13 at the Nebraska Agricultural Experiment Station, where the authors were, respectively, Chemist and Research Assistant in Chemistry.

<sup>2</sup> Reference is made by number to "Literature cited," p. 70-71.

determined by plant-house experiments, in which crop plants were grown in 6-foot cylinders and left unwatered until they matured or died of lack of water (2), appears to be practically coincident with the hygroscopic coefficient. Up to the present a method of estimating the water retentiveness in the field from one of the physical constants of the soil has not been developed. The laboratory experiments and field studies of the authors make it appear that, in the case of soils with hygroscopic coefficients between 14 and 3, this bears a rather simple relation to the hygroscopic coefficient, and that, in coarser soils, while it bears a much less simple relation, this is still one that may be experimentally determined. As the great majority of the tillable soils of dry-land regions fall within the limits of hygroscopicity mentioned, it would appear that, through the determination of the moisture content and the hygroscopic coefficient in the case of samples of the deeper subsoil, we could learn both the percentage of the physiologically important water and the departure of this from the maximum which the particular subsoil could retain.

The hygroscopic coefficient may be determined directly or more conveniently by one of the indirect methods that have been proposed (8, p. 73; 5, p. 410; 4, p. 531).

#### HISTORICAL REVIEW

The authors who maintain the theory "that water can rise to the surface from the deep layers by capillary action" are, as Rotmistrov has stated (23, p. 16), too numerous to name, but few of them offer any experimental evidence in support of the theory.

From field observations during unusually prolonged summer droughts Hall concluded that in certain soils the capillary rise of water might be as much as 200 feet (11, p. 94).

Mitscherlich, who has calculated the maximum possible elevation of water to be as high as 2 or 3 km. in heavy clays and loams (19, p. 192), considers this of no practical importance, on account of its extreme slowness of movement. From experiments with "the most varied soils" exposed for a 3-month period, during which they became appreciably altered by algæ, he observed no rise exceeding 0.8 meter and concluded that 1.5 meters from ground water may be regarded as the practical limit, so far as plants are concerned (20, p. 136).

In the case of one soil Tulaikow (24, p. 665) observed a rise of 135 cm. in 513 days, and the maximum had not yet been reached; while with three finer-textured soils the rise at the end of a year and a half had become stationary at 60 to 70 cm.

From field studies in Saskatchewan in 1904 and 1905 one of the authors concluded that in semiarid regions the roots go to the stored water in the subsoil instead of the latter being elevated to the surface foot by capillarity and that but comparatively little water which has once passed below the first foot is lost by evaporation (1, p. 42).

Leather (13, p. 105-106), from a study of the moisture in a fallow field at Pusa, India, during the dry season of 1906, concluded that—

during a dry period water moves upward toward the surface from a limited depth only; this limited depth increases with the period. Below this depth the water is stationary or possibly still draining downward.

In the Pusa soil he found the maximum distance that water moved upward during the period to be somewhat more than 3 feet and that eventually it was about 7 feet. While he did not determine the hygroscopicity of his soils or recognize in this a means of estimating the relative surfaces of the solid particles, he concluded that—

the relative water-retaining power of a soil after drainage has ceased is closely related to the total surface possessed by the solid particles, and it is probable that from a determination of the latter the water-holding capacity of soils may be ascertained.

Extreme views of the importance of the upward capillary movement have been expressed by Cameron (10) and McGee (16, 17). The former, mentioning that in humid areas the larger part of the water from rains returns to the surface, states that it sometimes does so "through distances of many feet" (10, p. 23). He assumes the upward capillary movement to be sufficient to bring to the surface annually more than sufficient potash and phosphoric acid to replace the amounts that would be removed by—

one ton per acre of dry crop containing one per cent. potash and 0.6 per cent. phosphoric acid (10, p. 77).

McGee has estimated that in the Great Plains of the United States the quantity of water which the deeper subsoil contributes to the growth of crops is not less than 6 inches annually and that by supplementing the local rainfall it suffices—

to render the land productive and habitable over a vast area which would otherwise be unproductive (17, p. 40); that it will move during the course of a year from a depth of say 10 feet; and that under favorable conditions of subsoil texture it will move during a term of years and progressively equalize the distribution of subsoil water through a depth of 30 or 35 feet (16, p. 11).

Widtsoe and McLaughlin (25, p. 230) have suggested the term "lento capillary point" to designate the moisture content of a soil at which capillary movements become very sluggish. They consider that it can not be defined with precision. In a field study of a soil very uniform in texture to a depth of 8 feet they found the moisture content to vary between about 10 and 18 per cent and the lento capillary point to lie between 12 and 13 per cent.

From experiments with crop plants grown in cylinders 6 feet deep, in which the hygroscopic coefficients of all the soils used were determined, one of the authors concluded that the stored moisture in the different depths of subsoil in the case of ordinary dry lands becomes available to

the plants by the roots being developed into these depths, but little water being elevated by capillarity from the zone below that traversed by the roots (2, p. 121).

Metal cylinders from 2 to 6 feet long and 4 inches in diameter were filled with a subsoil having a hygroscopic coefficient of 5.6, half with soil in a moister condition and half with it in a drier, allowed to stand for several months and then the change in moisture distribution determined. In most of the experiments the moisture content of the drier soil was approximately equal to the hygroscopic coefficient. When the water content of the moister soil was below about twice the hygroscopic coefficient, the capillary movement of water in any direction was slight; but when it was distinctly above this, there was a practically uniform movement from the moister into the drier soil (3, p. 286).

The work of Rotmistrov (23), near Odessa, covers a period of 15 years, 1895-1909. The ground water there lies at a depth of over a hundred feet and the soil is a Chernozem containing 3 to 5 per cent of organic matter. He assumes the "useless" (nonavailable) water at all depths in this soil to be about 10 per cent and attaches physiological importance to only the portion in excess of this. Moisture determinations, some 60,000 in all, were made at frequent intervals throughout the year at successive intervals of 5 or 10 cm., in some cases to a depth of 7 feet or more, both in clean fallows and under a great variety of crops. He found that when the subsoil is moist, the roots of annual crops penetrate to a depth of 2 to 5 feet and those of various perennials—alfalfa, trees, and shrubs—sometimes as deep as 60 feet. On the old plowed fields he found a permanently moist layer of subsoil extending from  $4\frac{1}{2}$  or  $5\frac{1}{2}$  feet to the water table, while on waste land occupied by weeds, etc., the permanently moist layer was encountered first at 14 to 30 feet. Above this is, first, a layer of subsoil which becomes moist or dry according to whether it is in fallow or crop, and, lastly, overlying the latter is the surface layer, varying from less than 2 to as much as 5 feet, which in every year becomes moist. He concludes that water percolating beyond a depth of 16 to 20 inches does not return to the surface except by way of the roots, the portion escaping the roots going down into the deeper layers at the rate of about 7 feet yearly.

Using glass tubes and wooden boxes, he carried out experiments with soil from the experimental field. Placing these in water, he observed a rise of less than 3 feet in three months. As the movement is so slow in the soil with water less than 3 feet below the surface, he concludes it will not move at all in the field where it is at a depth of over 100 feet.

Burr (9), from a 7-year study (1907 to 1913) of the total moisture in the first 3 to 15 feet of the comparatively uniform loessial soil on the table-land at North Platte, Nebr., where the water table is at a depth of over 200 feet, concludes that there is little upward movement of subsoil water, and that—

water supply by capillarity is not an important factor in crop production on Nebraska upland soils (9, p. 10).

The hygroscopic coefficients of the soil samples were not determined, but the mechanical analyses of eight sets of samples from the first 3 feet permit a calculation of these values by the Briggs and Shantz formula (8, p. 73); these vary from 6.1 to 8.5. From the extremes of moisture found he considers 16 to 18 per cent the maximum amount of water the soil can retain against gravity, and 7 to 8 per cent its minimum point of available water (9, p. 18-19). From this it appears that on that type of soil the hygroscopic coefficient is approximately the lower limit of available moisture, and that the maximum water content when downward movement ceases lies between 1.8 and 2.6 times the hygroscopic coefficient, if it is assumed that all the soil samples taken are sufficiently similar to justify such a comparison. This is in accord with our findings reported below.

#### CHARACTER OF SOILS USED

The soils were selected to represent some of the most important types of Nebraska, especially those of loessial origin, and not the whole range in texture from coarse sands with a hygroscopic coefficient less than 0.5 to clays with one in excess of 20. They include (Table I) six silt loams derived from the loess, five loams of residual origin, and one dune sand. Soil D is surface soil, Marshall silt loam, from the Experiment Station farm at Lincoln, and A the corresponding subsoil, taken from the third to the fifth foot. E and H similarly represent the surface soil and subsoil of the substation farm at Culbertson, and C and G corresponding depths of a prairie near McCook, both on Colby silt loam. Soils I, J, K, L, and M are from areas of residual soil mapped by the United States Bureau of Soils as belonging to the Sidney series (22, p. 58). I and K are surface soil and subsoil from a loam near Imperial, and M and L from a sandy loam near the same place. Soil J is a subsoil from the silt loam near Madrid, part of a bulk sample on which various studies (2, p. 46; 3, p. 249) have previously been reported. K and L are from the same depths as A, H, and G—viz, 3 to 5 feet—while J was from the fourth to the sixth foot.

Soil Q is a dune sand taken from a "blow-out" near Dunning, and is typical of the subsoil of the very extensive sand-hill region.

Soil B, the only soil from outside Nebraska included in the study, is from the Sulphur Spring Valley Dry-Land Experimental Farm, north of Douglas, Ariz. This was included because of its interesting conduct in the field. It was taken from the surface of a field which after two years of clean summer fallow, without bearing any crop at all, was found to contain no available water to a depth of 2 feet.



According to Ramann (21, p. 343),—

it is to be assumed that the capillary elevation of water is much more active in loess soils than with any other kind of soil.—Translation.

Six of the above soils are loess.

In Table I are reported the hygroscopic coefficient, the moisture equivalent (7, p. 140), the maximum water capacity as determined by the Hilgard method, and the total nitrogen.

TABLE I.—*Properties of soils used in the experiments*

Soil.	Total nitrogen.	Hygro- scopic coefficient.	Moisture equiva- lent.	Ratio of moisture equivalent to hygro- scopic coefficient.	Maximum water capacity.
	<i>Per ct.</i>				<i>Per ct.</i>
Loess soil from near Lincoln:					
Surface D.....	0.244	10.2	27.8	2.73	60.9
Subsoil A.....	.049	13.3	29.5	2.22	65.7
Loess soil from near McCook:					
Surface C.....	.104	10.5	24.1	2.30	63.7
Subsoil G.....	.029	8.2	21.2	2.59	55.4
Loess soil from near Culbertson:					
Surface E.....	.079	10.1	22.5	2.23	56.8
Subsoil H.....	.018	7.6	19.7	2.59	57.2
"Hard land" (residual) from near Im- perial:					
Surface I.....	.106	7.1	16.8	2.37	53.4
Subsoil K.....	.016	3.4	7.5	2.21	36.0
"Sandy land" (residual) from near Im- perial:					
Surface M.....	.077	3.3	7.9	2.39	34.2
Subsoil L.....	.023	3.4	7.2	2.12	31.0
"Hard land" from near Madrid:					
Subsoil J.....	.021	5.6	13.5	2.41	46.3
Dune sand from Dunning:					
Subsoil Q.....	.008	0.6	.....	.....	25.8
Arizona soil:					
Surface B.....	.088	12.9	25.8	2.00	60.3

#### METHOD OF FILLING AND OPENING CYLINDERS

In bringing a soil to the desired moisture content we placed a weighed quantity of the air-dried soil, of which the moisture content had previously been determined, upon a large sheet of oilcloth on the floor of the mixing room, and, while the mass was being shoveled over, added the calculated amount of water in small portions. The whole mass was then mixed thoroughly, first by shoveling, then by passing it twice through a swinging sieve of  $\frac{1}{4}$ -inch mesh, and finally by again shoveling; then it was immediately placed in a large covered can, allowed to stand for several days, again passed through the swinging sieve, returned to the can, and kept in this until transferred to the cylinders. The percentage of moisture thus secured was in the majority of cases from one-tenth to one-third higher than the hygroscopic coefficient, in the others it being nearer the desired amount.

In filling the cylinders the soil was added very slowly with constant tamping, care being taken to insure the firmness of that already in before adding more. Blows as uniform as possible were delivered by means of a tamper, for which we used a 2-inch rubber stopper on the end of a  $\frac{3}{8}$ -inch gas pipe 3 feet long. As the cylinders were being filled, three samples of each soil were taken for moisture determinations.

In such experiments as these the removal of exactly the desired depth of soil is an important operation, but in many cases a difficult one. Our practice with those of the metal cylinders in which there was no direct contact of the soil column under experiment with the subsoil mass was to place a cylinder on a table, and, by means of a can opener, to open it lengthwise, 6 to 12 inches at a time. The measured portions of the column of soil were then sliced off by means of a large spatula and placed in Mason jars, which were covered at once. With those of the cylinders open at the bottom and having the soil column in contact with the subsoil mass the successive layers were removed by a plate auger whose diameter was a little less than that of the cylinders. After the main portion of each section had been thus removed, the small residue next the cylinder wall was taken up with a spoonlike instrument. After all the samples had been secured from a cylinder, the contents of each jar were thoroughly mixed. The moisture determinations were made in an oven kept at  $110^{\circ}$  C., through which passed a rapid current of dry air. Precautions were taken to insure the thorough drying of all the samples.<sup>1</sup>

As was to be expected, all the surface soils, after being tamped into the cylinders, much resembled their condition in a well-prepared seed bed. The structure of the subsoils, with the exception of A, very closely resembled that found in excavations in the field. In the cylinders soil A had in all cases a granular structure, very different from that observed in the field. This, however, did not appear to influence seriously the movement of moisture, as will be seen below in the comparison of field data with those obtained in the laboratory experiments.

In most of the experiments duplicate cylinders were employed. The moisture conditions in the duplicates are so similar in all the soils, except the sand, Q, that we report only the average, no purpose being served by reporting the data from the two separately. With the sand the data on all the cylinders are reported. As illustrations of the degree of concordance with the soils other than the sand the data on two, C and I, are given in Table II.

The results in these experiments and in those previously reported (3) would make it appear an unprofitable expenditure of time, at least in preliminary studies, to use duplicate cylinders.

<sup>1</sup> Further particulars of the method have already been given in a report on cylinder experiments (2, p. 29).



TABLE II.—*Moisture conditions found on opening two typical pairs of duplicate cylinders, showing concordance of duplicates in the case of the finer-textured soils*

Depth of section.	Soil C.			Soil I.		
	Cylinder I.	Cylinder II.	Average.	Cylinder I.	Cylinder II.	Average.
<i>Inches.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
1.....	23.2	23.7	23.4	7.8	7.9	7.8
2.....	21.7	22.6	22.1	7.3	7.6	7.4
3.....	21.1	21.4	21.2	7.1	7.1	7.1
4.....	20.4	20.3	20.3	6.8	6.4	6.6
5.....	19.9	20.5	20.2	6.5	6.4	6.4
6.....	19.7	20.1	19.9	6.3	6.1	6.2
7.....	19.1	19.7	19.4	6.0	5.9	5.9
8.....	18.9	19.2	19.0	5.7	5.5	5.6
9.....	17.6	18.7	18.1	5.6	5.0	5.3
10.....	17.1	17.8	17.4	5.4	5.0	5.2
11.....	16.6	17.0	16.8	5.2	4.9	5.0
12.....	15.2	15.7	15.4	4.9	4.8	4.8
13.....	14.0	15.0	14.5	4.8	4.7	4.7
14.....	13.0	13.1	13.0	4.9	4.7	4.8
15.....	12.8	13.0	12.9	4.7	4.6	4.6
16.....	12.6	12.8	12.7	4.8	4.5	4.6
17.....	12.4	12.5	12.4	4.8	4.4	4.6
18.....	12.0	12.7	12.3	4.8	4.4	4.6
19.....	12.4	12.5	12.4	4.8	4.4	4.6
20.....	12.3	12.6	12.4	4.8	4.5	4.6
21.....	12.3	12.6	12.4	4.9	4.6	4.7
22.....	12.3	12.3	12.3	4.8	4.6	4.7
23.....	12.5	11.8	12.1	4.7	4.7	4.7
24.....	12.5	12.1	12.2	4.6	4.7	4.6
Average.....	15.9	16.3	16.1	5.5	5.3	5.4

#### FINAL WATER CONTENT WHEN SOIL COLUMN IS IN CAPILLARY CONNECTION WITH THE NATURAL SUBSOIL AND FULLY PROTECTED FROM EVAPORATION

A.—WITH A UNIFORM LOAM.—During the latter part of March, 1913, four cylinders 3 feet long, 6 inches in diameter, and open at both ends were placed in holes 8 inches in diameter bored in the loess floor of a greenhouse. The open space surrounding each cylinder was packed very tightly with moist subsoil in order to hold it firmly in place. The air-dried soil, J, was filled into the cylinders to a depth of 30 inches, thus bringing it to within 6 inches of the top, tamping it as above described (p. 33). Thus, direct capillary connection could be established between the soil of the cylinders and the natural subsoil, a loess with a hygroscopic coefficient of about 13. The moisture condition of the latter at a depth of 3 feet was similar to that in the fields near by. On the surface of the tamped soil a 2-inch layer of gravel was placed and 15 pounds of water added as rapidly as it soaked away, about 15 hours being required. As soon as all the water had been added, the tops of the cylinders were closed with tightly fitting covers; and a layer of moist soil, 8 inches in depth, was placed over all to prevent any loss by evaporation and also to protect the tops of the soil columns from the high temperature prevailing in the

greenhouse during the middle of the day (1, p. 26). As the weight of the air-dried soil in each cylinder was approximately 50 pounds, the added water was sufficient to have raised the moisture content to over 30 per cent if no seepage had occurred. During July, after the cylinders had been allowed 96 days in which to lose water by seepage, Cylinder IV was opened and the moisture content determined in the successive inch sections. The three others were opened at intervals of 8, 5, and 17 days, respectively. As the moisture content was no lower in the last three than in the first it would appear that equilibrium had been practically established by the time the first was opened. In Table III are reported the data on these as well as those on three cylinders (No. I to III) which, in an earlier experiment,<sup>1</sup> had been filled with the same soil and had been similarly treated (3, p. 280).

TABLE III.—Ratio of water content to hygroscopic coefficient in soil J, entirely protected from evaporation, but in capillary connection with the earth's soil mass. To the cylinders, each filled with approximately 50 pounds of air-dried soil, there was added 15 pounds of water, after which they were left from 31 to 126 days

Depth of section.	Cylinder No.							
	I (31 days).	II (44 days).	III (54 days).	IV (96 days).	V (104 days).	VI (109 days).	VII (126 days).	IV-VII (average 109 days).
Inches.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
1.....	3.1	3.3	3.1	2.5	3.0	2.6	2.6	2.6
2.....	3.1	3.2	3.0	2.4	2.8	2.6	2.5	2.6
3.....	3.1	3.2	3.1	2.3	2.7	2.5	2.5	2.5
4.....	3.2	3.2	3.1	2.4	2.6	2.5	2.5	2.5
5.....	3.3	3.2	3.1	2.4	2.6	2.5	2.5	2.5
6.....	3.2	.....	3.1	2.4	2.6	2.6	2.4	2.5
7.....	3.2	3.2	3.1	2.4	2.6	2.6	2.4	2.5
8.....	3.3	3.3	3.2	2.4	2.5	2.5	2.4	2.5
9.....	3.4	3.4	3.3	2.4	2.6	2.4	2.4	2.5
10.....	3.3	3.4	3.3	2.5	2.6	2.4	2.4	2.5
11.....	3.2	3.5	3.4	2.4	2.6	2.5	2.3	2.4
12.....	3.5	3.4	3.3	2.4	2.6	2.5	2.5	2.5
13.....	3.6	3.4	.....	2.4	2.6	2.5	2.4	2.5
14.....	3.6	3.5	3.4	2.4	2.6	2.4	2.4	2.5
15.....	3.7	3.6	3.4	2.4	2.5	2.4	2.5	2.5
16.....	3.6	3.4	3.5	2.3	2.5	2.4	2.6	2.4
17.....	3.7	3.6	3.5	2.3	2.5	2.4	2.5	2.4
18.....	3.7	3.9	3.5	2.3	2.6	2.4	2.5	2.4
19.....	3.7	3.7	3.5	2.3	2.6	2.4	2.5	2.4
20.....	3.7	3.7	3.6	2.4	2.6	2.4	2.5	2.5
21.....	3.8	3.8	3.7	2.3	2.5	2.3	2.5	2.4
22.....	4.0	4.1	3.8	2.3	2.6	2.4	2.5	2.4
23.....	4.2	3.9	3.8	2.3	2.5	2.4	2.4	2.4
24.....	4.2	3.9	3.8	2.3	2.5	2.5	2.5	2.4
25.....	4.2	3.9	4.1	2.3	2.5	2.5	2.5	2.4
26.....	4.4	4.3	4.2	2.3	2.5	2.5	2.5	2.4
27.....	4.5	4.5	4.2	2.3	2.5	2.5	2.5	2.4
28.....	4.5	4.3	4.2	2.5	2.4	2.5	2.4	2.5
29.....	4.6	4.3	4.5	2.5	2.5	2.5	2.4	2.5
30.....	4.8	4.3	4.6	2.5	2.5	2.5	2.5	2.5
Average.....	3.7	3.7	3.6	2.4	2.6	2.5	2.5	2.5

<sup>1</sup> The various experiments are reported in what appears a logical order for purposes of discussion rather than in the order in which they were performed.

In the latter group, exposed a year before, equilibrium had not been attained when the cylinders had been opened after periods of 31, 44, and 54 days, respectively, and the moisture content increased with the depth; while in the latter experiment, with a considerably longer exposure, the water was quite uniformly distributed throughout the 30 inches, varying only between 13.5 and 14.0, except in the first 2-inch section, in which it averaged 14.5 per cent.

The final average of the moisture content of the four cylinders of the later group (No. IV to VII) was 13.8, between 2.4 and 2.5 times the hygroscopic coefficient and approximately the same as the moisture equivalent, 13.5.

TABLE IV.—Ratio of moisture content to hygroscopic coefficient in soil J, entirely protected from evaporation and separated from the earth's soil mass by a 6-inch layer of coarse quartz sand or gravel. To the cylinders, filled with approximately 50 pounds of air-dried soil, there was added 15 pounds of water, after which the cylinders were left 126 days

Depth of section.	With sand.		With gravel.		Average, I-IV.
	Cylinder I.	Cylinder II.	Cylinder III.	Cylinder IV.	
Inches.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1.....	3.7	3.4	3.4	3.3	3.4
2.....	3.5	3.2	3.2	3.0	3.2
3.....	3.4	3.1	3.2	3.0	3.2
4.....	3.4	3.1	3.2	3.0	3.2
5.....	3.5	3.2	3.0	2.9	3.1
6.....	3.5	3.2	3.2	3.0	3.2
7.....	3.5	3.2	3.3	3.1	3.3
8.....	3.6	3.2	3.3	3.2	3.3
9.....	3.7	3.3	3.4	3.1	3.4
10.....	3.7	3.3	3.4	3.1	3.4
11.....	3.7	3.3	3.4	3.2	3.4
12.....	3.7	3.4	3.5	3.2	3.4
13.....	3.8	3.4	3.5	3.2	3.5
14.....	3.8	3.5	3.6	3.3	3.5
15.....	3.9	3.5	3.6	3.3	3.6
16.....	3.9	3.6	3.7	3.4	3.6
17.....	4.1	3.6	3.7	3.5	3.7
18.....	4.0	3.7	3.8	3.4	3.7
19.....	4.1	3.7	3.9	3.5	3.8
20.....	4.1	3.8	3.9	3.5	3.8
21.....	4.2	3.8	3.9	3.6	3.9
22.....	4.3	3.9	3.9	3.6	3.9
23.....	4.5	3.9	4.1	3.6	4.0
24.....	4.6	4.0	4.1	3.7	4.1
25.....	4.8	4.1	4.2	3.7	4.2
26.....	4.9	4.2	4.2	3.7	4.3
27.....	4.9	4.2	4.3	3.8	4.3
28.....	5.4	4.5	4.6	3.9	4.6
Average.....	4.0	3.6	3.7	3.3	3.6

B.—WITH A LOAM INTERRUPTED BY A GRAVEL OR SAND LAYER.—To determine the effect of an interrupting layer of coarse material, sand or gravel, four similar cylinders were filled with the same soil, J, but on the loess at the bottom of each cylinder there was first placed a 6-inch layer of quartz sand in the case of Cylinders I and II; and of gravel in III and IV. These coarse materials were tamped in uniformly, after which the

soil was added as above described and the same amount of water, 15 pounds, added. All four were opened at the end of 126 days. The moisture conditions are shown in Table IV. The moisture content was much higher, over 6 per cent on the average, than in the cylinders having no layer of coarse material. Further, in those with the sand or gravel the moisture in the soil column was not uniformly distributed, being about 6 per cent higher, just above the coarse layer, than near the surface. Neither the sand nor the gravel used in this experiment was as coarse as much of the material found naturally underlying arable soils.

C.—WITH LAYERS OF SIX DIFFERENT SOILS VARIOUSLY ARRANGED.—In this experiment we used soils selected to exhibit a wide range in hygroscopicity while still confining the set to soils representing important Nebraska types. These were placed in seven metal cylinders, open at both ends, 18 inches long, and 6 inches in diameter. The cylinders had been placed in a trench, 2 feet deep, dug in the loess floor of the greenhouse. In the case of each the sharp edge of the lower end of the cylinder was driven into the bottom of the trench to a depth of 2 inches. Then each cylinder was filled, in 2-inch layers, with six different soils, the layers being arranged differently in each (Table V). Thus, direct capillary connection was established between the natural subsoil and the soil column. Each soil in an air-dry condition was filled into the cylinder as above described. After the whole of the layer had been tamped in a small square piece of  $\frac{1}{4}$ -inch mesh galvanized-wire screen was placed on the surface before beginning the addition of the next layer, so that the dividing surfaces between the different layers of soil might be more readily recognized on opening the cylinders.

In the arrangement of the soils in Cylinders II to VII each soil appears once at the top, once at the bottom, and once in each intermediate position. In No. I the soils are arranged from top to bottom in the order of their hygroscopic coefficients, the soil with the lowest being at the top, while in No. VII the order is reversed.

TABLE V.—Arrangement of soil layers in the different cylinders, showing the soils used, their hygroscopic coefficients, and their moisture equivalents <sup>a</sup>

Depth.	SOILS						
	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
<i>Inches.</i>							
1-2.....	<b>Q</b>	<b>Q</b>	<b>L</b>	<b>J</b>	<b>H</b>	<b>D</b>	<b>A</b>
3-4.....	<b>L</b>	<b>A</b>	<b>Q</b>	<b>L</b>	<b>J</b>	<b>H</b>	<b>D</b>
5-6.....	<b>J</b>	<b>D</b>	<b>A</b>	<b>Q</b>	<b>L</b>	<b>J</b>	<b>H</b>
7-8.....	<b>H</b>	<b>H</b>	<b>D</b>	<b>A</b>	<b>Q</b>	<b>L</b>	<b>J</b>
9-10.....	<b>D</b>	<b>J</b>	<b>H</b>	<b>D</b>	<b>A</b>	<b>Q</b>	<b>L</b>
11-12.....	<b>A</b>	<b>L</b>	<b>J</b>	<b>H</b>	<b>D</b>	<b>A</b>	<b>Q</b>

<sup>a</sup> The heavy lines indicate the position of the sand layers.

TABLE V.—Arrangement of soil layers in the different cylinders, showing the soils used, their hygroscopic coefficients, and their moisture equivalents—Continued

Depth.	HYGROSCOPIC COEFFICIENT						
	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
<i>Inches.</i>							
1-2.....	0.6	0.6	3.4	5.6	7.6	10.2	13.3
3-4.....	3.4	13.3	0.6	3.4	5.6	7.6	10.2
5-6.....	5.6	10.2	13.3	0.6	3.4	5.6	7.6
7-8.....	7.6	7.6	10.2	13.3	0.6	3.4	5.6
9-10.....	10.2	5.6	7.6	10.2	13.3	0.6	3.4
11-12.....	13.3	3.4	5.6	7.6	10.2	13.3	0.6

Depth.	MOISTURE EQUIVALENT						
	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
<i>Inches.</i>							
1-2.....	1.5	1.5	7.2	13.5	19.7	27.8	29.5
3-4.....	7.2	29.5	1.5	7.2	13.5	19.7	27.8
5-6.....	13.5	27.8	29.5	1.5	7.2	13.5	19.7
7-8.....	19.7	19.7	27.8	29.5	1.5	7.2	13.5
9-10.....	27.8	13.5	19.7	27.8	29.5	1.5	7.2
11-12.....	29.5	7.2	13.5	19.7	27.8	29.5	1.5

After placing in the cylinder the six layers as described we added a 2-inch layer of gravel to the top of the column and then applied 7 pounds of water. As the soil column in each cylinder weighed not more than 15 pounds, this amount of water was sufficient to insure saturation, as it would have raised the moisture content to 50 per cent or more if the soil could have retained so much. As soon as all the water had been added, the tops of the cylinders were closed with tightly fitting covers and, as in sections A and B, a layer of moist soil was placed over all in order to prevent any evaporation and to protect the cylinders from the heat of the sun.

The cylinders, filled on September 7, 1912, were allowed to remain undisturbed for 69 days, when they were removed from the trench and opened, placing each on its side, cutting the metal from top to bottom and flattening out the metal sheet, thus exposing the whole soil column to observation. Each layer was carefully detached by means of a spatula, freed of all material belonging to the layers above and below, and thoroughly mixed for the moisture determination. The moisture conditions found on opening the cylinders are shown in the accompanying tables, VI giving the percentages of total water, VII that of free water, VIII the ratio of the total water to the hygroscopic coefficient, and IX the ratio of the total water to the moisture equivalent. The



term "free water" is used to designate the difference between the total water and the hygroscopic coefficient (14, p. 66) and is not synonymous

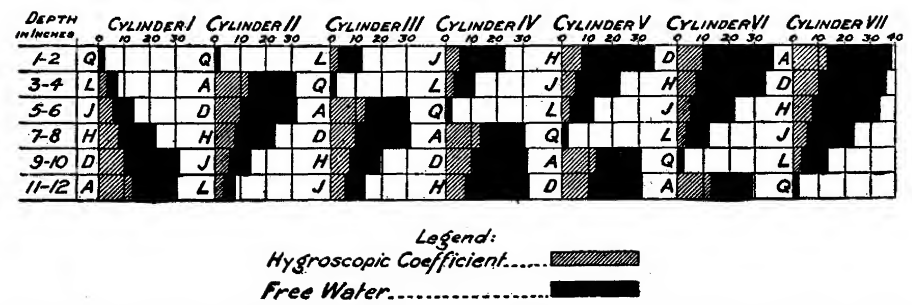


FIG. 1.—Diagram showing the relation of the percentage of water retained to the hygroscopic coefficient and the influence of an interrupting layer of coarse sand (soil Q). The seven soil columns, consisting of 2-inch layers of six different soils variously arranged, were saturated and then allowed to stand for 69 days protected from evaporation and in contact with the natural subsoil mass.

with "gravitational water" as it is employed by some (15, p. 207). The data are presented graphically in figure 1.

TABLE VI.—Total water in the successive soil layers<sup>a</sup>

Depth.	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1-2.....	2.2	2.2	12.2	22.9	36.0	37.6	38.3
3-4.....	7.7	31.5	2.4	11.2	22.6	34.2	36.0
5-6.....	13.4	31.9	30.8	2.5	12.4	22.1	33.9
7-8.....	22.2	23.1	31.6	30.6	2.4	12.1	23.7
9-10.....	31.8	13.8	19.8	31.8	30.1	2.3	13.4
11-12.....	30.4	7.7	13.4	23.2	31.2	29.5	2.7

<sup>a</sup> The heavy lines indicate the position of the sand layers.

TABLE VII.—Free water in the successive soil layers

Depth.	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1-2.....	1.7	1.7	8.8	17.3	28.4	27.4	25.0
3-4.....	4.3	18.2	1.0	7.8	17.0	26.6	25.8
5-6.....	7.8	21.7	17.7	2.0	9.0	16.5	26.3
7-8.....	14.6	15.5	21.4	17.3	1.9	8.7	18.1
9-10.....	21.6	8.2	12.2	21.6	16.8	1.8	10.0
11-12.....	17.1	4.3	7.8	15.6	21.0	16.2	2.1

TABLE VIII.—Ratio of water content to hygroscopic coefficient in the successive soil layers<sup>a</sup>

## A.—DATA ARRANGED ACCORDING TO DEPTH

Depth.	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
<i>Inches.</i>							
1-2.....	4.4	4.4	3.6	4.1	4.7	3.7	2.9
3-4.....	2.1	2.4	4.8	3.3	4.0	4.5	3.5
5-6.....	2.4	3.1	2.3	5.0	3.6	4.0	4.5
7-8.....	2.9	3.0	3.1	2.3	4.8	3.5	4.2
9-10.....	3.1	2.5	2.6	3.1	2.3	4.6	3.9
11-12.....	2.3	2.1	2.4	3.1	3.1	2.2	5.4

## B.—DATA ARRANGED ACCORDING TO SOILS

Soil No.	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
Q.....	4.4	4.4	4.8	5.0	4.8	4.6	5.4
L.....	2.1	2.1	3.6	3.3	3.6	3.5	3.9
J.....	2.4	2.5	2.4	4.1	4.0	4.0	4.2
H.....	2.9	3.0	2.6	3.1	4.7	4.5	4.5
D.....	3.1	3.1	3.1	3.1	3.1	3.7	3.5
A.....	2.3	2.4	2.3	2.3	2.3	2.2	2.9

<sup>a</sup> The heavy lines indicate the position of the sand layers.TABLE IX.—Ratio of water content to the moisture equivalent in the successive soil layers<sup>a</sup>

## A.—DATA ARRANGED ACCORDING TO DEPTH

Depth.	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
<i>Inches.</i>							
1-2.....	1.5	1.5	1.7	1.7	1.8	1.3	1.3
3-4.....	1.1	1.1	1.6	1.6	1.7	1.7	1.3
5-6.....	1.0	1.1	1.0	1.7	1.7	1.6	1.7
7-8.....	1.1	1.2	1.1	1.0	1.6	1.7	1.8
9-10.....	1.1	1.0	1.0	1.1	1.0	1.5	1.9
11-12.....	1.0	1.1	1.0	1.1	1.1	1.0	1.8

## B.—DATA ARRANGED ACCORDING TO SOILS

Soil No.	Cylinder No.						
	I.	II.	III.	IV.	V.	VI.	VII.
Q.....	1.5	1.5	1.6	1.7	1.6	1.5	1.8
L.....	1.1	1.1	1.7	1.6	1.7	1.7	1.9
J.....	1.0	1.0	1.0	1.7	1.7	1.6	1.8
H.....	1.1	1.2	1.0	1.1	1.8	1.7	1.7
D.....	1.1	1.1	1.1	1.1	1.1	1.3	1.3
A.....	1.0	1.1	1.0	1.0	1.0	1.0	1.3

<sup>a</sup> The heavy lines indicate the position of the sand layers.



The ratio of the total water to the hygroscopic coefficient is strikingly similar for all the soils where the sand layer did not interrupt their connection with the natural subsoil mass, it varying only from 2.1 to 3.1. For soil J it is similar to that found in section A above.

From the tables it will be seen that while it apparently makes no difference as to the order of the soil layers, with the exception of the dune sand Q, the interposition of this has in all cases greatly increased the amount of water held by the soils in the layers above it. The ratio of the total water to hygroscopic coefficient varies from 4.4 to 5.4 for the sand. Soil L shows a ratio of 2.1 in Cylinders I and II when below the sand, but of 3.3 to 3.9 in the others, where the sand underlies it. The ratios for J are 2.4 to 2.5 against 4.0 to 4.2, for H 2.6 to 3.1 against 4.5 to 4.7, for D 3.1 against 3.5 to 3.7, and for A 2.2 to 2.4 against 2.9. Thus, where the sand layer overlies the finer-textured soil the ratio of the retained moisture in the latter to the hygroscopic coefficient varies only between 2.1 and 3.1, while where it underlies the latter the ratio is from 0.4 to 2.1 higher.

The concordance of the retained moisture with the moisture equivalent (Table IX) where the sand layer does not interrupt is even much closer than its relation to the hygroscopic coefficient, the ratio being 1.0 to 1.2.

The sand layer Q is in all cases low in moisture compared with the amount found when water has been added to the surface of a 2-foot column of the same soil, it varying from 2.2 to 2.7 per cent; whereas in the latter it lies between 3.4 and 6.0 per cent, even 83 days after 1 inch of water has been added (p. 54).

Mitscherlich (20, p. 143) has pointed out that a very thin layer of loam in a sandy subsoil may markedly retard the movement of water through the latter. It is evident from the above that, conversely, thin layers of coarse sand or gravel may retard the movement of water in a loam.

#### FINAL WATER CONTENT WHEN DIFFERENT AMOUNTS OF WATER ARE ADDED TO THE TOP OF A COLUMN OF AIR-DRIED SOIL

In this experiment we used 8 pairs of cylinders, each 18 inches long, 6 inches in diameter, closed at the bottom, and provided with a tightly fitting cover. Twelve inches of air-dried soil D, containing 3.0 per cent of water, was tamped into each of 10, and a volume of water, equivalent to 1, 2, 3, 4, and 5 inches of rain, respectively, was added in small portions as rapidly as it was absorbed. The 6 other cylinders were similarly filled with the soil J in an air-dry condition, carrying 2.3 per cent of water, and treated with 1, 2, and 3 inches of water. Then the surface was covered with an inch layer of gravel, the cylinders covered, placed in a covered pit, and left undisturbed for 47 days, at the end of which they were opened, and the water determined in inch sections. The data

on the ratio of moisture content to the hygroscopic coefficient are reported in Table X.

TABLE X.—Ratio of moisture content to hygroscopic coefficient in cylinders 47 days after 1 to 5 inches of water had been applied to the surface of columns of air-dried soil. The hygroscopic coefficient of soil D was 10.2 and of J 5.6, and the initial moisture content 3 and 2.3 per cent, respectively

Depth.	Soil D.					Soil J.		
	1 in.	2 in.	3 in.	4 in.	5 in.	1 in.	2 in.	3 in.
<i>Inches.</i>								
1.....	1.9	2.7	3.0	3.7	.....	1.9	2.7	4.0
2.....	1.9	2.6	2.8	3.3	3.9	1.8	2.6	3.7
3.....	1.7	2.6	2.7	3.2	3.9	1.8	2.5	3.6
4.....	1.1	2.6	2.7	3.2	3.8	1.8	2.5	3.6
5.....	.8	2.4	2.7	3.2	3.8	1.7	2.4	3.5
6.....	.7	2.4	2.7	3.1	3.7	1.6	2.3	3.5
7.....	.6	1.8	2.6	3.1	3.6	1.3	2.3	3.5
8.....	.6	1.1	2.5	3.0	3.6	1.0	2.2	3.4
9.....	.5	.8	2.3	3.0	3.5	.9	2.1	3.1
10.....	.5	.7	2.0	2.9	3.5	.8	2.0	2.9
11.....	.5	.7	1.4	2.7	3.5	.8	2.0	2.8
12.....	.5	.7	1.2	2.6	3.5	.7	2.0	2.9

In all the cylinders the moisture content of all portions of the soil column had been raised either by capillary movement or by the passage of water vapor through the air. With soil D, capillary movement had affected the water content to a depth of 4 inches with 1 inch of water, to 8 inches with 2, and to 12 inches with 3, while with the still larger amounts the soil had become very moist throughout the whole length of the column. With soil J, where 1 inch of water had been added, the moisture was distributed uniformly to a depth of 4 or 5 inches, below which it decreased very rapidly; but, where twice as much had been added, the moistened soil extended to the bottom of the column; and with 3 inches the whole column had become very moist.

With the larger quantities of water, 4 and 5 inches with soil D and 3 inches with J, the downward movement had been arrested by the bottom of the cylinder; but the exposure was not sufficiently long to permit the moisture distribution to attain equilibrium.

#### FINAL WATER CONTENT WHEN TOP OF SOIL COLUMN IS EXPOSED TO EVAPORATION

Two water-tight cylinders, 6 inches in diameter, 3 feet deep, and closed at the bottom, were sunk in holes in the greenhouse, as described above; but the tops of the cylinders were placed level with the surface of the greenhouse floor and left open. On March 8, 1912, these cylinders, after being used in a similar experiment, previously reported (3, p. 283),

were refilled with soil J, carrying 15.2 per cent of water, and allowed to stand for 185 days before opening. The soil was tamped in as usual, this being continued to the top in the case of one and to within 1 inch of the top in the case of the other, the last inch in which was filled with air-dry soil. This, not being tamped, formed a shallow mulch. Both cylinders were so situated that the sun could shine on them nearly all day long; but, as whitewash was used on the windows, the maximum daily temperatures were not much higher than those in the open air. Thus, the mean maximum temperature in the plant house from July 5 to the end of the month was 93° F., while that in the open air was 91°. The corresponding average daily mean temperatures for the same period were 84° and 79°, respectively. For the month of August the mean maximum temperature in the plant house was 84° and in the open air 75°. While in the greenhouse the temperatures were somewhat higher, there was much less wind movement; and accordingly the soil in the cylinders was exposed to somewhat the same, or slightly less, drying influences that it would have experienced had it been in the open air but entirely protected from rainfall.

During the first three months of the experiment the cylinder with the compact surface was daily examined for the presence of minute cracks, and both cylinders were examined for the presence of crevices around the walls. As soon as cracks or crevices appeared they were filled with dry soil from the surface, so that all water lost would have to pass through the surface layer, instead of part of it escaping through such ventilating fissures. As, long before the experiment was concluded, cracks and crevices had ceased to form, examinations were made much less frequently after the third month.

The distribution of moisture found at the end of six months is shown in Table XI. The loss of water was greatest at the surface and least toward the bottom of the cylinders. All portions had suffered a loss of 4 per cent or more. A uniform upward movement, similar to that mentioned in the case of the earlier experiments, is to be observed in the case of the portion below the first 12 inches. The soil below this depth became as dry as in the earlier experiment (Table XII), but the latter had been exposed less than half as long. It is probable that the lower portion of the soil in both experiments had practically ceased to lose water. The loss of water from the two cylinders was similar, averaging for each 5.7 per cent. The final moisture content of the portion of the column below the twelfth inch varied only from 10 per cent at the top to 11 at the bottom, with an average of 10.7, or 1.9 times the hygroscopic coefficient.

TABLE XI.—Loss of water from a uniform soil, J, protected from all loss at the sides and below, but fully exposed to evaporation at the surface. Both cylinders were filled on March 8 with soil containing 15.2 per cent of water and left for 185 days

Depth of section.	Water content on opening cylinder.			Loss of water in 185 days.		
	Cylinder I, surface mulched.	Cylinder II, surface compact.	Average.	Cylinder I, surface mulched.	Cylinder II, surface compact.	Average.
<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1.....	3.7	3.7	3.7	11.5	11.5	11.5
2.....	4.5	3.8	4.2	10.7	11.4	11.1
3.....	5.8	5.9	5.9	9.4	9.3	9.4
4.....	6.9	6.8	6.9	8.3	8.4	8.4
5.....	7.3	7.7	7.5	7.9	7.5	7.7
6.....	7.8	7.7	7.8	7.4	7.5	7.5
7.....	8.3	8.5	8.4	6.9	6.7	6.8
8.....	9.0	9.2	9.1	6.2	6.0	6.1
9.....	9.2	9.4	9.3	6.0	5.8	5.9
10.....	9.2	9.7	9.5	6.0	5.5	5.8
11.....	9.7	9.8	9.8	5.5	5.4	5.5
12.....	9.7	10.0	9.9	5.5	5.2	5.3
13.....	10.0	10.1	10.1	5.2	5.1	5.2
14.....	10.1	10.0	10.1	5.1	5.2	5.2
15.....	10.1	10.4	10.3	5.1	4.8	5.0
16.....	10.0	10.3	10.2	5.2	4.9	5.1
17.....	10.5	10.5	10.5	4.7	4.7	4.7
18.....	10.6	10.4	10.5	4.6	4.8	4.7
19.....	10.6	10.7	10.7	4.6	4.5	4.6
20.....	10.6	10.6	10.6	4.6	4.6	4.6
21.....	10.9	10.6	10.8	4.3	4.6	4.5
22.....	11.0	10.7	10.9	4.2	4.5	4.4
23.....	10.9	10.6	10.8	4.3	4.6	4.5
24.....	11.1	10.7	10.9	4.1	4.5	4.3
25.....	11.0	10.7	10.9	4.2	4.5	4.4
26.....	11.1	11.1	11.1	4.1	4.1	4.1
27.....	11.1	11.0	11.1	4.1	4.2	4.2
28.....	11.1	11.0	11.1	4.1	4.2	4.2
29.....	11.1	11.0	11.1	4.1	4.2	4.2
30.....	11.0	11.0	11.0	4.1	4.2	4.2
Average....	9.5	9.5	9.5	5.7	5.7	5.7

The data obtained from a similar experiment on the same soil but with an initial moisture content of only 11.8 and exposed for shorter periods, 22 and 77 days, are reported in Table XII (3, p. 283). In this case no loss of water from the bottom of the soil column was shown during the first three weeks; but at the end of 11 weeks it amounted to 1 per cent, and the amount remaining in the lowest 18-inch portion of the column also averaged 10.7, the same as remained after the longer exposure of the more moist soil.

TABLE XII.—Loss of water from a uniform soil, J, protected from all loss at the sides and below, but fully exposed to evaporation at the surface. All cylinders were filled on February 14 with soil containing 11.8 per cent of water

Depth of section.	Water content on opening cylinder.				Loss of water.			
	March 7.		May 1.		In 22 days.		In 77 days.	
	Cylinder I (Surface mulched).	Cylinder II (Surface packed).	Cylinder III (Surface mulched).	Cylinder IV (Surface packed).	Cylinder I.	Cylinder II.	Cylinder III.	Cylinder IV.
Inches.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
1.....	3.0	3.3	2.2	2.2	8.8	8.5	9.6	9.6
2.....	4.4	6.5	2.2	2.4	7.4	5.3	9.6	9.4
3.....	7.4	8.2	3.8	4.6	4.4	3.6	8.0	7.2
4.....	8.8	8.6	5.6	6.2	3.0	3.2	6.2	5.6
5.....	9.1	9.1	6.8	7.4	2.7	2.7	5.0	4.4
6.....	9.5	9.4	7.4	7.8	2.3	2.4	4.4	4.0
7.....	9.5	10.0	7.8	8.8	2.3	1.8	4.0	3.0
8.....	9.7	10.1	8.1	8.6	2.1	1.7	3.7	3.2
9.....	9.9	10.1	8.3	8.9	1.9	1.7	3.5	2.9
10.....	10.1	10.3	9.0	9.1	1.7	1.5	2.8	2.7
11.....	10.3	10.5	9.1	9.4	1.5	1.3	2.8	2.4
12.....	10.4	10.6	9.2	9.5	1.4	1.2	2.6	2.3
13.....	10.6	10.8	9.7	9.5	1.5	1.0	2.1	2.3
14.....	10.9	10.9	10.0	9.8	1.5	.9	1.8	2.0
15.....	11.0	11.1	10.0	10.1	1.2	.7	1.8	1.7
16.....	11.2	11.2	9.9	10.0	.9	.6	1.9	1.8
17.....	11.0	11.4	9.8	9.9	.8	.4	2.0	1.9
18.....	11.1	11.4	9.9	9.9	.6	.4	1.9	1.9
19.....	11.1	11.4	10.0	10.7	.8	.4	1.8	1.1
20.....	11.1	11.4	10.0	10.6	.7	.4	1.8	1.2
21.....	11.2	11.4	10.1	10.2	.7	.4	1.7	1.2
22.....	11.4	11.4	10.2	10.3	.7	.4	1.7	1.6
23.....	11.4	11.4	10.4	10.3	.6	.4	1.6	1.5
24.....	11.5	11.4	10.5	10.4	.4	.4	1.4	1.5
25.....	11.4	11.4	10.6	10.7	.4	.4	1.3	1.4
26.....	11.5	11.5	10.7	10.8	.3	.3	1.2	1.1
27.....	11.4	11.5	10.7	10.7	.4	.3	1.1	1.0
28.....	11.5	11.5	10.9	10.7	.3	.3	1.1	1.1
29.....	11.5	11.6	10.9	10.8	.3	.2	.9	1.1
30.....	11.5	11.7	11.0	10.8	.3	.1	.9	1.0
31.....	11.5	11.7	11.0	11.0	.3	.1	.8	1.0
32.....	11.5	11.7	11.0	10.8	.3	.1	.8	1.0
33.....	11.5	11.8	11.0	10.8	.3	.0	.8	1.0
34.....	11.8	11.8	11.0	10.8	.0	.0	.8	1.0
35.....	11.8	11.8	11.0	10.8	.0	.0	.8	1.0
36.....	11.8	11.8	11.0	10.8	.0	.0	.8	1.0
Average.....	10.3	10.6	9.2	9.4	1.5	1.2	2.6	2.4

The movement from the portion of the subsoil below the twelfth inch evidently becomes exceedingly slow after the moisture content has fallen to a point approximately twice the hygroscopic coefficient. It should not be overlooked, however, that the point at which movement upward into the very dry surface has practically ceased is appreciably below that at which it occurred with the downward movement into the moist mass of the natural subsoil (Table XII), the ratio being 1.9 or 2.0 with the former and 2.4 with the latter. However, if even this close a concordance is found to hold in the field, the knowledge of the ratio would be very useful. Applying it, we should expect that a subsoil such as J with



a hygroscopic coefficient of 5.6 and situated in the second to the fifth foot below the surface would, after prolonged heavy rains, if protected from transpiration losses, as by clean fallowing, lose water by downward movement until it approached 14.0 per cent, while if exposed for six months to hot, rainless weather it would still carry not less than about 11 per cent, the available water then varying between approximately 8 and 5 per cent.

TABLE XIII.—Loss of water from a uniform soil, D, protected from all loss at the sides and bottom, but fully exposed to evaporation at the surface. All of the cylinders were filled on September 2, No. I and II with soil containing 24.4 per cent and No. III and IV with soil containing 30.6 per cent of water. All were opened at the end of 78 days

Depth of section.	Water content on opening cylinder.						Loss of water.	
	Cylinder I.	Cylinder II.	Average.	Cylinder III.	Cylinder IV.	Average.	Cylinder I-II.	Cylinder III-IV.
Inches.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
1.....	5.7	4.3	5.0	.....	.....	.....	19.4	.....
2.....	8.3	7.2	7.7	7.3	7.3	7.3	16.7	23.3
3.....	11.8	12.3	12.0	11.6	11.6	11.6	12.4	19.0
4.....	16.0	14.9	15.4	16.0	14.8	15.4	9.0	15.2
5.....	17.3	16.4	16.8	17.5	17.3	17.4	7.6	13.2
6.....	17.7	17.0	17.3	19.2	17.2	18.2	7.1	12.4
7.....	18.1	17.7	17.9	19.8	18.8	19.3	6.5	11.3
8.....	19.3	18.2	18.7	20.3	19.2	19.7	5.7	10.9
9.....	19.7	19.0	19.3	20.8	19.9	20.3	5.1	10.3
10.....	20.0	19.0	19.5	21.1	20.7	20.9	4.9	9.7
11.....	20.3	19.8	20.0	22.0	21.3	21.6	4.4	9.0
12.....	20.4	20.6	20.5	22.4	21.7	22.0	3.9	8.6
13.....	21.5	20.5	21.0	23.0	22.5	22.7	3.4	7.9
14.....	21.7	20.6	21.1	23.3	22.8	23.0	3.3	7.6
15.....	22.1	20.7	21.4	23.5	22.8	23.1	3.0	7.5
16.....	22.0	20.9	21.4	23.6	23.6	23.6	3.0	7.0
17.....	22.4	21.4	21.9	23.5	24.0	23.7	2.5	6.9
18.....	22.6	21.2	21.9	24.2	24.5	24.3	2.5	6.3
19.....	22.3	22.5	21.9	24.0	24.9	24.4	2.5	6.2
20.....	22.6	22.5	22.5	25.0	24.7	24.8	1.9	5.8
21.....	22.0	22.0	22.0	25.0	24.9	24.9	2.4	5.7
22.....	22.3	22.1	22.2	25.1	25.1	25.1	2.2	5.5
23.....	22.3	22.3	22.3	25.2	25.6	25.4	2.1	5.2
24.....	22.3	22.4	22.3	25.0	25.3	25.1	2.1	5.5
25.....	22.6	22.4	22.5	25.2	25.1	25.1	1.9	5.5
26.....	22.6	22.3	22.4	25.3	24.9	25.1	2.0	5.5
27.....	22.6	22.4	22.5	25.5	25.6	25.5	1.9	5.1
28.....	23.0	22.4	22.7	25.6	25.4	25.5	1.7	5.1
29.....	23.0	22.5	22.7	25.9	25.8	25.8	1.7	4.8
30.....	22.9	22.6	22.7	25.8	25.5	25.6	1.7	5.0
31.....	23.1	22.7	22.9	25.5	25.2	25.3	1.5	5.3
32.....	22.6	22.8	22.7	25.6	25.2	25.3	1.7	5.3
33.....	22.9	23.2	23.0	25.9	25.4	25.6	1.4	5.0
34.....	23.0	23.2	23.1	24.8	25.5	25.6	1.3	5.0
35.....	22.8	22.8	22.8	26.0	25.7	25.8	1.6	4.8
36.....	22.6	22.6	22.6	26.0	(a)	26.0	1.8	4.6
Average:								
1-3.....	8.6	7.9	8.2	9.4	9.4	9.4	16.2	21.1
1-6.....	12.8	12.0	12.4	14.3	13.6	13.9	12.0	16.6
1-9.....	14.8	14.1	14.4	16.6	15.8	16.2	9.9	14.4
1-12.....	16.2	15.5	15.8	18.0	17.2	17.6	8.6	12.9
13-24.....	22.2	21.5	21.8	24.2	24.2	24.2	2.6	6.4
25-36.....	22.8	22.6	22.7	25.6	25.4	25.5	1.7	5.1

<sup>a</sup> 26.0 used to obtain average.



A similar experiment was conducted with the silt-loam surface soil, D, which is rich in organic matter and has a hygroscopic coefficient of 10.2, and so is in sharp contrast with the residual subsoil J, practically devoid of organic matter and having a hygroscopic coefficient of only 5.6. The cylinders were similar to those used with J. Two, No. I and II, were filled with soil D, containing 24.4 per cent, and the others, No. III and IV, with the same soil, carrying 30.6 per cent of water. All were left with a compact surface for 11 weeks.

The losses of moisture in the former were confined chiefly to the surface foot, the final ratios in the second and third feet being 2.1 and 2.2, respectively, as compared with the initial ratio of 2.4. In the case of the two cylinders with the moister soil, with an initial ratio of 3.0, there was a distinct loss from all depths, the final ratio in the second and third feet being 2.4 and 2.5, respectively. The ratio in the upper half of a 12-inch column of this soil, to the surface of which in an air-dry condition 2 or 3 inches of water had been applied, after which it had been allowed to stand for 47 days, protected from evaporation, lay between 2.4 and 3.0 (Table X).

DISTRIBUTION OF MOISTURE WHEN EQUILIBRIUM HAS BEEN  
ATTAINED AFTER ADDING WATER TO THE SURFACE OF A COLUMN  
WHOSE MOISTURE CONTENT IS APPROXIMATELY EQUAL TO THE  
HYGROSCOPIC COEFFICIENT

In this experiment we used all 13 soils mentioned in Table I. In the case of each soil two galvanized-iron cylinders 2 feet long, 4 inches in diameter, and provided with bottoms and tight-fitting covers, were filled, as described above, with the soil having a moisture content approximately equal to the hygroscopic coefficient. To permit the escape of air as the water was being added, small holes had been made in the bottoms. The soil was tamped in until even with the rim, smoothed off, and covered with a metal tray, which was left on until all the cylinders had been filled.

In the case of each of the soils, except the dune sand Q, the initial water content was approximately equal to the hygroscopic coefficient and the amount of water added to the surface was sufficient to raise the average moisture content of all the soil in the cylinder to 1.5 times the hygroscopic coefficient, it varying according to the soil and the initial moisture content of this from 0.27 inch to 2.12 inches. To the sand Q, which was in an air-dry condition, the added water amounted to 0.5 inch in two cylinders and to 1.0 inch in two others.

To make more uniform the initial penetration of the water, the cylinders used were inverted in the flat-bottomed metal trays, the desired amount of water then added and allowed to rise into the soil by capillarity until all or nearly all had been absorbed, after which they were placed right side up. Where with the coarsest soils a few drops of water remained in the tray, these were added to the surface after the cylinder had

been inverted. Any soil adhering to the tray was transferred to the cylinder. The cover was then put on, and both the opening between this and the cylinder wall and the holes in the bottom were sealed with paraffin. The cylinders were weighed and transferred to a basement room in which the temperature was practically constant. There they were placed in a large box and covered to a depth of 8 inches with moist soil to prevent any sudden or extreme fluctuations in temperature and also any drying of the soil in the cylinders, if by any chance openings should develop, as, for example, by the cracking of the paraffin seal.

The cylinders were left undisturbed for 10 weeks, and most of them for 4 to 6 weeks longer. As soon as each was removed from the box, it was weighed, in order to determine whether there had been any change in weight during its stay in the box. In the case of none did the scales, sensitive to half an ounce, show any gain or loss. As the cylinders were removed from the box, moist soil was put in their place. All had been

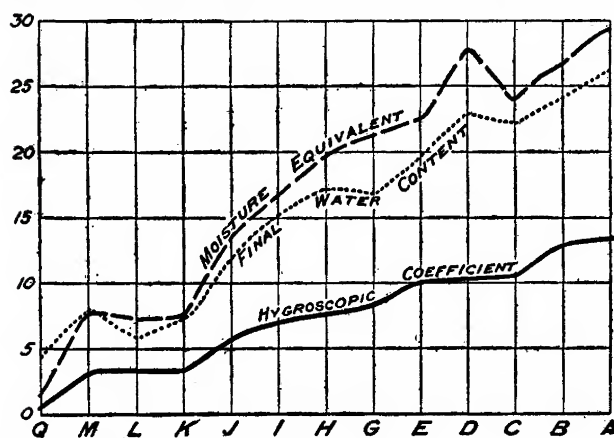


FIG. 2.—Diagram showing relationship between the final water content and both the hygroscopic coefficient and the moisture equivalent.

standing the distribution of water was but little affected by leaving them longer undisturbed (3, p. 270).

The distribution of the moisture at the end of this period for the 12 loams is given in Table XIV. That in the sand Q is treated separately. It will be seen that, except with B and G, the lower foot section had gained but little in moisture during the 69 to 110 days. Almost all the soils showed a very slight increase, even as far as the lowest portion of the cylinder; but only with B and G was this really distinct as deep as the twenty-first inch. The maximum depth affected in any case may be considered as the twenty-fourth inch. The final moisture content of the surface 3-inch section lay between 1.7 and 2.4 times the hygroscopic

coefficient, the maximum in all cases being found in the surface inch. This relationship is shown in Table XV. Where the initial ratio was 1 or 1.1, the final ratio in the surface 3-inch section varied from 1.7 to 2.3, that in the lowest 3-inch section remaining as low as 1.1 to 1.2. Where

placed in the box on the same day, but in some instances not all of those removed at the same time could be opened on the same day. In such cases the unopened cylinders were left in the constant temperature room until such time as they were opened. Previous experiments with soil J had shown that after two months'

the initial ratio was as high as 1.3, the final ratio of the surface 3-inch section was similar to the above and that in the lowest section varied from 1.3 to 1.5.

The final moisture content of the surface layer in these loams bears an even closer relationship to the moisture equivalent than to the hygroscopic coefficient, being in general about nine-tenths of the latter value, the ratio varying from 0.80 to 1.01 (Table XVI and figure 2). As will be pointed out below, this is not the case with the sand Q and it would appear that with sands in general there would be a departure, increasing with the coarseness of the texture. However, the great majority of agricultural soils have a texture no coarser than that of K, L, or M.

TABLE XIV.—*Distribution of water in cylinders filled with soil containing a percentage of water approximately equal to the hygroscopic coefficient. To the surface of each enough water was applied to raise the total water content to one and a half times the hygroscopic coefficient, after which the cylinders were allowed to stand from 68 to 110 days*

Item.	Soil No.											
	A.	B.	C.	D.	E.	G.	H.	I.	J.	K.	L.	M.
Soil:												
Hygroscopic coefficient	13.3	12.9	10.5	10.2	10.1	8.2	7.6	7.1	5.6	3.4	3.4	3.3
Initial water, per cent.	13.2	13.1	12.0	13.4	9.9	8.0	9.0	8.4	5.6	4.3	4.5	4.3
Initial ratio	1.0	1.0	1.1	1.3	1.0	1.0	1.2	1.2	1.0	1.3	1.3	1.3
Water applied, inches	2.11	2.12	1.21	.90	1.42	1.58	1.28	.60	.89	.33	.27	.33
Moisture equivalent	29.5	25.8	24.1	27.8	22.5	21.2	19.7	16.8	13.5	7.5	7.2	7.9
Time, days	110	69	100	110	100	106	102	102	70	100	68	100
Final water content (per cent):												
Depth—												
1 inch	27.5	25.5	23.4	24.1	20.5	17.4	18.0	16.3	12.2	7.8	6.2	8.7
2 inches	26.1	23.7	22.1	22.9	19.3	16.7	16.6	15.1	11.7	7.4	5.8	8.0
3 inches	25.1	23.5	21.2	21.6	19.0	16.3	16.7	14.0	11.3	7.1	5.5	7.3
4 inches	24.7	23.3	20.3	20.8	18.5	16.2	16.3	13.6	10.8	6.6	5.5	7.2
5 inches	24.3	22.9	20.2	20.2	18.6	16.0	15.9	13.6	10.5	6.4	5.4	6.6
6 inches	23.7	23.1	19.9	19.0	18.3	15.7	15.7	13.2	10.3	6.2	5.0	5.7
7 inches	22.9	23.1	19.4	18.0	18.0	15.6	15.5	12.7	10.0	5.9	5.1	5.0
8 inches	22.0	22.7	19.0	16.8	17.7	15.4	15.1	11.8	9.6	5.6	4.9	4.7
9 inches	20.8	22.4	18.1	15.9	16.9	15.0	14.8	10.8	9.5	5.3	4.6	4.7
10 inches	19.5	21.8	17.4	14.8	16.7	14.6	14.6	9.9	9.1	5.2	4.6	4.7
11 inches	17.6	21.4	16.8	14.0	16.2	14.4	14.2	9.5	8.6	5.0	4.3	4.6
12 inches	16.5	20.8	15.4	13.6	15.3	14.2	13.7	9.2	8.2	4.8	4.4	4.6
13 inches	15.3	20.4	14.5	13.4	14.8	14.0	13.2	8.9	7.5	4.7	4.2	4.7
14 inches	14.5	20.1	13.0	13.2	14.1	13.6	12.5	8.0	7.3	4.8	4.2	4.7
15 inches	14.0	19.6	12.9	13.1	13.2	13.3	12.3	8.8	6.6	4.6	4.1	4.7
16 inches	13.6	19.0	12.7	13.1	12.3	13.1	11.3	8.7	6.2	4.6	4.1	4.6
17 inches	13.5	18.3	12.4	13.1	11.7	12.9	10.7	8.7	6.0	4.6	4.2	4.7
18 inches	13.3	17.5	12.3	13.1	11.4	12.3	10.3	8.6	6.0	4.6	4.2	4.6
19 inches	13.2	16.9	12.4	13.1	11.0	11.8	9.6	8.7	5.7	4.6	4.4	4.7
20 inches	13.2	15.7	12.4	13.2	10.9	11.2	9.7	8.7	5.7	4.6	4.4	4.7
21 inches	13.1	15.0	12.4	13.2	10.8	10.7	9.7	8.7	5.7	4.7	4.4	4.8
22 inches	13.1	14.5	12.3	13.3	10.6	10.2	9.8	8.8	5.7	4.7	4.5	4.8
23 inches	13.0	14.5	12.2	13.2	10.5	9.9	9.7	8.8	5.9	4.7	4.4	4.8
24 inches	13.0	13.5	12.2	13.3	10.5	10.0	9.7	8.7	5.7	4.6	4.1	4.8
Average, 1 to 24 inches	18.1	19.9	16.0	15.9	14.8	13.8	13.1	10.6	8.2	5.4	4.7	5.3
Average, 1 to 12 inches	22.6	22.8	19.4	18.5	17.9	15.6	15.6	12.5	10.1	6.1	5.1	6.0
Average, 13 to 24 inches	13.6	17.1	12.6	13.2	11.8	11.9	10.7	8.7	6.2	4.7	4.3	4.7
Change in water content (per cent):												
1 to 12 inches	9.4	9.7	7.4	5.1	8.0	7.6	6.6	4.1	4.5	1.8	.6	1.7
13 to 24 inches	.4	4.0	.6	— .2	1.9	3.9	1.7	.3	.6	.4	.2	.4

TABLE XV.—Ratio of final water content to the hygroscopic coefficient from 68 to 110 days after water had been applied to the surface of the soil column

Soil.	Water added.	Initial ratio.	Final ratio.							
			1 to 3 inches.	4 to 6 inches.	7 to 9 inches.	10 to 12 inches.	13 to 15 inches.	16 to 18 inches.	19 to 21 inches.	22 to 24 inches.
	<i>Inches.</i>									
A.....	2.11	1.0	2.0	1.8	1.7	1.3	1.1	1.0	1.0	1.0
B.....	2.12	1.0	1.9	1.8	1.8	1.7	1.6	1.4	1.2	1.1
C.....	1.21	1.1	2.1	1.9	1.8	1.6	1.3	1.2	1.2	1.2
D.....	.90	1.3	2.2	2.0	1.7	1.4	1.3	1.3	1.3	1.3
E.....	1.42	1.0	1.9	1.8	1.8	1.6	1.4	1.2	1.1	1.0
G.....	1.58	1.0	2.0	2.0	1.9	1.7	1.7	1.6	1.4	1.2
H.....	1.28	1.2	2.3	2.1	2.0	1.9	1.6	1.4	1.3	1.3
I.....	.60	1.2	2.1	1.9	1.7	1.3	1.3	1.2	1.2	1.2
J.....	.89	1.0	2.1	1.9	1.7	1.5	1.3	1.1	1.0	1.0
K.....	.33	1.3	2.2	1.9	1.6	1.5	1.4	1.4	1.4	1.4
L.....	.27	1.3	1.7	1.6	1.4	1.3	1.2	1.2	1.3	1.3
M.....	.3	1.3	2.4	2.0	1.4	1.4	1.4	1.4	1.4	1.5

TABLE XVI.—Ratio of final water content in the surface 3-inch section of loams to the moisture equivalent

Soil No.	Final water content, 1 to 3 inches.	Moisture equivalent.	Ratio.	Soil No.	Final water content, 1 to 3 inches.	Moisture equivalent.	Ratio.
A.....	26.2	29.5	0.88	H.....	17.1	19.7	0.87
B.....	24.2	25.8	.94	I.....	15.1	16.8	.90
C.....	22.2	24.1	.92	J.....	11.7	13.5	.87
D.....	22.9	27.8	.82	K.....	7.4	7.5	1.00
E.....	19.6	22.5	.87	L.....	5.8	7.2	.86
G.....	16.8	21.2	.80	M.....	8.0	7.9	1.01

In the ratio of final water content to hygroscopic coefficient the extremes are shown by L, a subsoil, and M, the corresponding surface soil. The same soils also show almost the extremes in the ratio of final water content to moisture equivalent, in both cases the ratio being higher with M.

The conduct of the three soils K, L, and M, all from the same locality and similar in hygroscopicity, was striking, L retaining much less and M much more water than K. This behavior appeared so exceptional that the experiment with these was repeated two years later at the Minnesota Experiment Station, using duplicate cylinders in which soils K, L, and M had initial moisture contents of 4.4, 4.3, and 4.1 per cent, respectively. To each we added 0.33 inch of water, after which they were stored in a pit for 132 to 144 days. The resulting data are so nearly identical with those given in Table XIV that no purpose would be served by reporting them. This makes it certain that the differences between K, L, and M are not simply the result of unavoidable errors of experiment.



DISTRIBUTION OF MOISTURE WHEN EQUILIBRIUM HAS BEEN ATTAINED AFTER ADDING WATER TO THE BASE OF A COLUMN WHOSE MOISTURE CONTENT IS APPROXIMATELY EQUAL TO ITS HYGROSCOPIC COEFFICIENT

This experiment was similar to the preceding, except that the water was applied to the base of the column instead of to the surface. Similar cylinders were filled with the same soils, in the same manner, and at the same time, and were then placed upright in the small metal trays and the same amounts of water added as in the preceding experiment. The water was quickly absorbed through the small holes. The covers had been left off to permit the ready escape of the air expelled from the soil by the ascending water, the intention being to have these replaced as soon as the water had been absorbed, for which only a very short time was necessary, and then have both the opening between the cover and the cylinder wall and the holes in the bottom at once sealed with paraffin. Through a misunderstanding the covers were not placed on the cylinders, but all the latter being placed close together, each still resting in its tray, were covered with a large sheet of oilcloth and left in the basement room. Three days later, when the error was discovered, it was found that the oilcloth had been moved so that some of the cylinders were uncovered; but no one employed about the laboratory knew when the oilcloth had been disturbed or, accordingly, how long some of the cylinders had been exposed to evaporation. None had been weighed after being filled; but the weights of the empty cylinders, those of the different soils used, the initial moisture content, and the amount of the added water permitted a close calculation of what the weight of each should have been, provided no loss through evaporation had occurred. All the cylinders were at once covered and weighed. Those filled with soils A, D, and H had suffered the greatest loss, it having been, as closely as could be estimated, sufficient to lower the moisture content of a section of the soil column 6 to 8 inches deep by 2 or 3 per cent. As we decided to allow the cylinders to stand and determine the final moisture conditions, all were sealed and stored in the box of soil in the basement room and otherwise handled like those in the parallel experiment.

The duplicate cylinders of each of the loams showed a similar distribution of moisture; and, hence, only the averages are reported in Table XVII. In every cylinder the final moisture content of the uppermost section was found to be below the initial water content, the upward movement of water during the period of 74 to 115 days not having been sufficient to compensate for the loss into the still atmosphere in the darkened room during two and one-half days. This striking evidence of the extreme slowness of the upward movement of water under moisture conditions of the subsoil that resemble those met in dry-land fields with the water table far below that surface may possibly give the experiment an even greater value than it would have possessed had the misunderstanding not occurred.

TABLE XVII.—Distribution of water in cylinders filled with soil containing a percentage of water approximately equal to the hygroscopic coefficient. To the bottom of each soil column enough water was added to raise the average water content to one and one-half times the hygroscopic coefficient, after which the cylinders were allowed to stand from 74 to 115 days

Item.	Soil No.											
	A.	B.	C.	D.	E.	G.	H.	I.	J.	K.	L.	M.
Soil:												
Hygroscopic coefficient.	13.3	12.9	10.5	10.2	10.1	8.2	7.6	7.1	5.6	3.4	3.4	3.3
Initial water, per cent <sup>a</sup> .	13.2	13.1	12.0	13.4	9.9	8.0	9.0	8.4	5.6	4.3	4.5	4.3
Initial ratio.	1.0	1.0	1.1	1.3	1.0	1.0	1.2	1.2	1.0	1.3	1.3	1.3
Water applied, inches.	2.11	2.12	1.21	.90	1.42	1.58	1.28	.60	.89	.33	.27	.33
Moisture equivalent.	29.5	25.8	24.1	27.8	22.5	21.2	19.7	16.8	13.5	7.5	7.2	7.9
Time.....days.	115	74	107	115	107	107	107	107	107	107	73	107
Final water content (per cent):												
Depth—												
1 inch.....	10.9	12.6	9.9	9.8	8.0	8.0	7.5	6.5	4.7	3.7	3.1	2.9
2 inches.....	11.3	12.6	10.2	10.5	8.4	8.4	7.6	6.9	4.9	4.0	3.1	2.8
3 inches.....	11.7	13.5	10.3	11.1	8.9	8.9	8.0	7.3	5.1	4.1	3.1	3.6
4 inches.....	11.8	15.1	10.7	11.7	9.2	9.4	8.4	7.6	5.2	4.3	3.5	3.4
5 inches.....	12.1	16.6	11.4	12.1	9.6	10.4	8.9	7.8	5.3	4.4	3.5	3.6
6 inches.....	12.4	18.3	11.8	12.5	10.1	11.1	9.5	8.2	5.6	4.4	4.0	3.9
7 inches.....	12.6	18.9	12.2	12.5	10.7	11.5	10.0	8.4	5.7	4.3	3.9	4.1
8 inches.....	13.0	19.6	12.4	12.8	11.3	11.5	10.7	8.7	5.9	4.6	3.9	4.2
9 inches.....	13.3	20.1	12.8	12.9	12.5	13.1	11.5	8.7	6.5	4.7	3.9	4.4
10 inches.....	14.0	20.4	13.5	13.2	13.8	13.4	12.5	8.8	7.3	4.7	4.3	4.5
11 inches.....	15.1	20.7	14.9	13.4	14.6	13.9	13.1	9.0	7.9	5.0	4.3	4.4
12 inches.....	16.5	21.2	16.1	13.7	15.7	14.4	13.5	9.3	8.3	5.3	4.4	4.5
13 inches.....	18.2	21.9	17.0	14.5	16.2	14.8	14.2	9.7	8.9	5.2	4.5	4.6
14 inches.....	19.9	22.3	17.8	15.1	17.0	15.0	14.4	10.4	9.3	5.4	4.7	4.5
15 inches.....	21.2	22.9	18.8	16.2	17.2	15.5	15.0	11.4	9.9	5.6	4.9	4.8
16 inches.....	22.3	23.0	19.3	17.4	17.3	15.8	15.2	12.3	10.0	5.9	5.2	5.0
17 inches.....	23.3	23.3	19.7	18.8	18.0	16.3	15.7	12.9	10.2	6.3	5.7	5.7
18 inches.....	23.8	23.4	20.1	19.6	18.3	16.6	16.0	13.1	10.5	6.6	5.6	6.5
19 inches.....	24.2	23.7	20.5	20.8	18.8	17.0	16.4	14.0	10.3	6.9	5.8	7.0
20 inches.....	24.9	24.1	21.0	21.6	19.2	17.3	16.8	14.4	10.5	7.5	6.1	7.3
21 inches.....	25.3	24.0	21.1	22.4	19.4	17.3	17.1	14.6	10.7	7.4	5.9	8.1
22 inches.....	25.7	24.1	21.7	22.8	19.4	17.4	17.1	15.0	11.7	7.4	6.3	8.0
23 inches.....	25.9	24.1	21.8	23.5	19.6	17.5	17.1	15.0	11.9	7.4	6.4	8.2
24 inches.....	26.2	24.2	22.0	23.5	19.4	17.4	17.1	15.0	11.9	7.2	6.2	8.0
Average, 1 to 24 inches.....	18.2	20.4	16.1	16.0	14.7	13.8	13.0	10.6	8.3	5.5	4.7	5.2
Average, 1 to 12 inches.....	12.9	17.5	12.2	12.2	11.1	11.2	10.1	8.1	6.0	4.5	3.8	3.9
Average, 13 to 24 inches.....	23.4	23.4	20.1	19.7	18.3	16.5	16.0	13.2	10.5	6.6	5.6	6.5

<sup>a</sup> Some moisture was lost from the surface of the soil columns immediately after they were filled.

The distribution of moisture is just the reverse of that in the parallel experiment (Table XIV) with the same series of soils, the effect of the influence of gravity having disappeared during the prolonged exposure. The similarity is best shown by a comparison of the ratio of the final moisture content to the hygroscopic coefficient (Table XVIII), in which the data are so arranged as to facilitate comparison with those in Table XV. The similarity is striking, the soils which in the one show a low final ratio in the sections of the soil column nearest the point of application of the water showing a similar ratio in the other. The movement had been so alike in both experiments that the final distribution of the moisture appears independent of the direction through which the water had had to move, and we might regard the moisture conditions shown by either, aside from the dry sections in the latter experiment due to the error in procedure, to represent those for the particular soil, no matter what angle the axis of the column may make with the perpendicular.



TABLE XVIII.—*Ratio of final water content to the hygroscopic coefficient 74 to 115 days after water had been applied to the base of the soil column*

Soil No.	Water added.	Initial ratio. <sup>a</sup>	Final ratio of—							
			24 to 22 inches.	21 to 19 inches.	18 to 16 inches.	15 to 13 inches.	12 to 10 inches.	9 to 7 inches.	6 to 4 inches.	3 to 1 inches.
	<i>Inches.</i>									
A.....	2.11	1.0	2.0	1.9	1.8	1.5	1.1	1.0	0.9	0.8
B.....	2.12	1.0	1.9	1.9	1.8	1.7	1.6	1.5	1.3	1.0
C.....	1.21	1.1	2.1	2.0	1.9	1.7	1.4	1.2	1.1	1.0
D.....	.90	1.3	2.3	2.1	1.8	1.5	1.3	1.2	1.1	1.0
E.....	1.42	1.0	1.9	1.9	1.8	1.7	1.5	1.1	1.0	.8
G.....	1.58	1.0	2.1	2.1	2.0	1.8	1.7	1.6	1.3	1.0
H.....	1.28	1.2	2.2	2.2	2.1	1.9	1.7	1.4	1.2	1.0
I.....	.60	1.2	2.1	2.0	1.8	1.5	1.3	1.2	1.1	1.0
J.....	.89	1.0	2.1	1.9	1.8	1.7	1.4	1.1	.9	.9
K.....	.33	1.3	2.2	2.1	1.8	1.6	1.5	1.3	1.3	1.2
L.....	.27	1.3	1.9	1.7	1.6	1.4	1.3	1.2	1.1	.9
M.....	.33	1.3	2.4	2.2	1.7	1.4	1.4	1.3	1.1	1.0

<sup>a</sup> In the 1- to 3-inch section, and, in some of the soils, also in the next section, the initial moisture content was somewhat lower than this value.

The initial moisture content in the above parallel experiments is similar to that found in the subsoil traversed by the plant roots when the plants have just died or have begun to die from lack of moisture (2, p. 122). From these experiments it would appear safe to conclude that the water from any soil layer in which the ratio is not above 1.7 will not appreciably affect the moisture content of the soil at a distance of 12 inches or more, even during a period of three months. Even the maximum that remains in a subsoil in contact with the earth's soil mass after downward movement has ceased appears able to affect the moisture content to but a slightly greater distance during such a period. It would appear that with loam soils, such as those employed, the maximum ratio to be expected near the point of application, several months after the water had been added, would be between 1.6 and 2.5.

Under field conditions evaporation might prevent this ratio being found close to the surface, except for short periods following rain or irrigation, but at a distance of a foot or two it is to be expected, and, if not disturbed by the invasion of plant roots, may long persist.

When the ratio lies much below 1.0, the more moist soil seems to exert an influence upon the drier soil through a greater distance, which is to be attributed not to a movement of water along the surface of the soil grains but to evaporation of moisture into the air from the soil having a ratio in excess of 1.0 and its absorption from the resulting saturated atmosphere by the soil with a ratio below 1.0 (3, p. 259).

#### EXCEPTIONAL CONDUCT OF DUNE SAND

The distribution of water in coarse sands takes place quite differently from that in the loams above dealt with. Using the dune sand Q, with an initial content of 0.2 per cent of water, we added 0.50 inch in the case of five cylinders and 1.0 inch in the case of five others. All had been

filled and otherwise treated like those with the 12 loam soils, except that at the conclusion of the experiment the soil was removed in 3-inch instead of 1-inch sections (Table XIX). One cylinder of each group was opened at the end of 29, 63, and 78 days, respectively, while the remaining two were opened after 83 days. The distribution of moisture was very much the same at the end of 29 days as after 34 days more; but with the heavier application of water an appreciable downward movement took place between the sixty-third and the eighty-third day.

The 0.5 inch of water distinctly raised the moisture content of the sand to a depth of 6 inches and the 1-inch application to a depth of 12 to 15 inches. The surface 3-inch layer showed a final ratio of 8 to 10.

At the same time that water was added to the tops of the above 10 cylinders of dune sand it was applied to the base of the soil columns in two others filled at the same time, 0.5 inch to one and 1.0 inch to the other. At the end of 83 days both were opened. The moisture content of the five sections 1 to 12, 13 to 15, 16 to 18, 19 to 21, and 22 to 24 inches was 0.3, 0.5, 0.7, 4.4, and 7.1 per cent, respectively, in the former and 0.4, 0.5, 1.0, 5.6, and 12.1 per cent in the latter. In neither does the capillary movement seem to have extended beyond the ninth inch. The increase in moisture content in the higher sections is to be attributed to the movement of moisture through the soil atmosphere.

TABLE XIX.—*Distribution of water in cylinders filled with dune sand, they being allowed to stand from 29 to 83 days after 0.5 inch of water had been added to those in experiment A and 1.0 inch to those in B*

A.—0.5 INCH OF WATER ADDED

Depth of section.	Cylinder I (29 days).	Cylinder II (63 days).	Cylinder III (78 days).	Cylinder IV (83 days).	Cylinder V (83 days).	Average.
<i>Inches.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1-3.....	6.2	5.7	6.2	5.8	6.0	6.0
4-6.....	2.6	2.2	2.6	2.7	3.0	2.6
7-9.....	.4	.5	.5	.5	.5	.5
10-12.....	.2	.2	.4	.4	.4	.3
13-15.....	.2	.3	.5	.4	.4	.4
16-18.....	.2	.2	.3	.4	.4	.3
19-21.....	.2	.2	.4	.4	.3	.3
22-24.....	.2	.3	.3	.3	.4	.3
Average, 1-24.....	1.3	1.2	1.6	1.5	1.4	1.3
Average, 1-12.....	2.3	2.1	2.4	2.4	2.5	2.4
Average, 13-24.....	.2	.2	.4	.4	.4	.3

B.—1.0 INCH OF WATER ADDED

1-3.....	5.8	5.5	4.1	3.4	4.5	4.7
4-6.....	4.6	4.9	4.4	3.6	4.0	4.3
7-9.....	4.3	4.0	3.9	3.6	3.8	3.9
10-12.....	4.1	3.6	3.9	3.7	3.9	3.8
13-15.....	.6	.6	2.9	3.6	3.3	2.2
16-18.....	.6	.3	.7	1.5	.6	.7
19-21.....	.6	.4	.8	.5	.5	.6
22-24.....	.6	.3	.3	.4	.5	.4
Average, 1-24.....	2.6	2.4	2.6	2.5	2.7	2.6
Average, 1-12.....	4.7	4.5	4.1	3.6	4.1	4.1
Average, 13-24.....	.6	.4	1.2	1.5	1.2	1.0

The coarse sand exerts a retarding influence upon the movement of moisture through its own mass as well as upon that from overlying layers of finer texture.

The similarity in conduct of the soils with hygroscopic coefficients between 3.3 and 13.3 and the entirely different behavior of the sand with one of 0.6 make the conduct of the soils with values between 0.6 and 3.3 of great interest. As we included no representatives of these in the laboratory experiments, our conclusions in regard to them are based only upon field studies (p. 64).

#### RATIOS FOUND IN FIELD STUDIES

The published data on the total moisture content of soils of almost every degree of coarseness or fineness of texture, and determined under all kinds of weather, crop, and tillage conditions, are almost innumerable; but, as almost none of these are accompanied by statements of the hygroscopic coefficients of the samples or of any physical constants which would permit the calculation of these (8, p. 56; 6, p. 842; 4, p. 351), the data do not admit of testing the generalizations drawn from the above-described laboratory experiments. Occasionally an author has made such determinations on a few of the samples and then assumed that the subsoils were so uniform that these would apply to all. This assumption is correct in only exceptional places, and even then it should be first experimentally justified instead of the question being merely glossed over by the statement that the soils are characterized by great uniformity. For this reason we have to confine ourselves to our own field studies, carried out between 1907 and 1913 and as yet unpublished. All of these, we hope, will soon appear in another paper. As the determinations of the water content of each of the field samples was followed by one of the hygroscopic coefficient, the ratios are available in each case. In most instances the subsoil moisture had been more or less exhausted by plant roots and showed a ratio of between 1.0 and 1.5; such data are not of interest in the present study.

Our data on soils with hygroscopic coefficients lying between 3.0 and 14.0 are very numerous; those on the sands with a coefficient below 1.0 limited; and those on fine sands with coefficients between 3.0 and 1.1 very scanty. Of the first group we select only some worked out in the most detail.

It is scarcely permissible to compare the ratios of the final water content to the moisture equivalent, unless the latter value has been directly determined in the case of the samples in question, because the relation of this constant to the hygroscopic coefficient is quite variable (6, p. 845).

A.—COARSE SANDS.—Our data from field studies of the moisture in coarse sands are not numerous, but what we have are in accord with the results of the cylinder experiments. Table XX shows the conditions in the Nebraska sand hills in 1911 and 1912. Alternating hilltops and

basins, as well as scattered blow-outs, are characteristic features of this region. The last are almost bare of vegetation, and, hence, present conditions favorable to the subsoil carrying the maximum amount of water. The hilltops have a more scanty stand of plants than the basins; and, as a consequence, we should expect a smaller loss of water through transpiration and less likelihood of a low moisture content in the subsoil. All samples were composites from three borings, 10 to 30 feet apart.

The hygroscopic coefficient in all cases is low, 0.3 to 1.1. The moisture content, while low, was from 3 to 12 times the hygroscopic coefficient, the ratio in most cases lying between 4 and 8.

TABLE XX.—Ratio of water content of Nebraska dune sands under natural conditions to the hygroscopic coefficient

Depth.	Near Valentine, Aug. 21, 1911.			Near Thedford, Dec. 2, 1912.				Halsey Forest Reserve, Dec. 3, 1912.					
	Hill-top.	Basin.	Blow-out.	Blow-out 1.	Blow-out 2.	Blow-out 3.	Blow-out 4.	Blow-out 1.		Blow-out 2.		Hill-top.	Basin.
								Side.	Bottom.	Side.	Bottom.		
Feet.													
1.....	4.6	4.4	6.5	4.7	5.3	4.0	4.1	3.1	3.1	2.7	2.4	2.9	3.3
2-3.....				4.6	7.5	3.2	4.3	2.9	3.7	2.9	3.4	2.9	4.0
4-5.....				6.0	10.0	6.6	6.0	3.3	4.1	2.9	3.2	2.6	4.1
6.....	4.5	2.7	6.1	6.5	8.5	6.6	8.0	2.9	4.1	3.1	2.7	1.7	3.7
HYGROSCOPIC COEFFICIENT													
1.....	0.8	0.8	0.8	0.8	1.0	0.9	0.10	0.5	0.3	0.4	0.5	0.5	0.6
2-3.....				.8	1.0	1.0	1.0	.8	.4	.4	.5	.5	.6
4-5.....				.8	.8	.9	1.1	.4	.4	.4	.6	.5	.7
6.....	.8	.9	.8	1.0	.8	1.1	1.1	.4	.4	.4	.6	.4	.7
RATIO OF TOTAL WATER TO HYGROSCOPIC COEFFICIENT													
1.....	5.7	5.5	8.1	5.9	5.3	4.4	4.1	6.2	10.3	6.7	4.8	5.8	5.5
2-3.....				5.7	7.5	3.2	4.3	3.6	9.2	7.2	6.8	5.8	6.7
4-5.....				7.5	12.5	7.3	5.5	8.2	10.2	7.2	5.3	5.2	6.9
6.....	5.6	3.0	7.6	6.5	10.6	6.0	7.3	7.2	10.2	7.8	4.5	4.2	5.3

A study in the abandoned Pope olive orchard, described by Mason (18, p. 17), 5 miles south of Palm Springs station, at the southern end of the Colorado Desert in California, furnished the data reported in Table XXI. After four months of hot, rainless weather, a rain, amounting, at the nearest United States Weather Bureau station, 5 miles north, to 1.90 inches, had fallen six days before the sampling. Pits adjacent to old trees were dug, exposing the subsoil below the lowest point to which the moisture from the recent rain had penetrated, and samples were taken from the walls of these pits.

The data show that the water had traveled downward only after raising the ratio to from 5 to 10.

It thus appears that, in the case of coarse sands, we may in general expect to find the water content as high as from 5 to 10 times the hygro-

scopic coefficient, unless it has been reduced by transpiration or evaporation. As capillarity in sands elevates water but a very short distance, the proportion of the rainfall available for transpiration by deep-rooted plants may be surprisingly large, as during the growing season the run-off is likely to be negligible, most of the water quickly penetrating beyond the influence of surface evaporation; and finally the further penetration to any great depth is much delayed, thus allowing time for its withdrawal by the roots.

TABLE XXI.—*Ratio of water content to hygroscopic coefficient in sandy soils and subsoil in an abandoned orchard near Palm Springs, Cal., on October 10, 1912*

PERCENTAGE OF TOTAL WATER			
Depth.	Pit I.	Pit II.	Pit III.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1 to 12 inches.....	6.3	5.4	4.7
Second foot.....	8.6	6.0	4.9
Third foot.....	7.0	6.1	4.6
Fourth foot.....	6.4	.7	.7
Fifth foot.....	1.1	.7	1.3
HYGROSCOPIC COEFFICIENT			
2 to 12 inches.....	0.6	0.7	0.7
Second foot.....	.6	.7	.9
Third foot.....	.7	.7	.5
Fourth foot.....	.6	.7	.5
Fifth foot.....	.6	.6	.7
RATIO OF TOTAL WATER TO HYGROSCOPIC COEFFICIENT			
2 to 12 inches.....	10.5	7.7	6.7
Second foot.....	14.3	8.6	5.4
Third foot.....	10.0	8.7	9.2
Fourth foot.....	10.7	1.0	1.4
Fifth foot.....	1.8	1.2	1.9

B.—FINER-TEXTURED SOILS.—Data from a field study in the summer of 1912 permit a close comparison with the data from the laboratory experiments on soils A and D. A was a bulk composite from the fourth to fifth foot taken from an excavation being made for a new building on the Experiment Station grounds. Three hundred yards away a considerable area of subsoil had recently been exposed in preparing a railroad grade, 3 or 4 feet of surface material having been removed. Throughout the season we kept this exposed subsoil free of weeds, and at frequent intervals took samples for the determination of moisture in the different inch sections of the surface foot and less frequently in the foot sections to a depth of 6 feet. The samples employed in the case of the inch sections were from duplicate borings, 10 to 20 feet apart, using a soil auger 4 inches in diameter. D was a bulk sample of the surface 6 inches of soil taken from one side of a long cultivated field adjacent to the exposed



subsoil. This field was planted to corn in 1912; but an area 50 feet square, near the center of the field and less than 100 yards from both the exposed subsoil and the place where soil D had been collected, was kept free from all vegetation, but was cultivated the same as though it had been in corn. Moisture determinations parallel to those on the exposed subsoil were made here.

TABLE XXII.—*Hygroscopic coefficients, nitrogen, and organic carbon of the inch sections of surface foot in the areas near the Nebraska Experiment Station used for a detailed moisture study*

FALLOW FIELD

Depth.	Hygroscopic coefficient.					Nitro- gen.	Organic carbon.
	Set I.	Set II.	Set III.	Set IV.	Aver- age.		
<i>Inches.</i>						<i>Per ct.</i>	<i>Per ct.</i>
1.....	8.7	8.5	8.8	7.9	8.5	0.237	.....
2.....	8.5	8.2	8.7	8.6	8.5	.235	.....
3.....	8.5	8.9	9.3	9.0	8.9	.233	2.78
4.....	8.2	8.5	9.2	9.0	8.7	.233	.....
5.....	8.5	8.7	8.7	9.2	8.8	.253	.....
6.....	8.7	8.7	9.2	8.5	8.8	.251	.....
7.....	8.8	9.4	9.3	8.0	8.9	.239	.....
8.....	8.7	9.9	10.5	8.4	9.4	.211	2.27
9.....	9.3	9.6	10.5	9.2	9.7	.188	.....
10.....	10.3	10.0	11.2	9.3	10.2	.171	.....
11.....	11.1	10.5	11.6	9.8	10.8	.164	.....
12.....	11.2	11.3	12.6	10.5	11.4	.154	1.86
Average, 1-6.....	8.5	8.6	9.0	8.7	8.7	.....	.....
Average, 7-12.....	9.9	10.1	11.0	9.2	10.1	.....	.....
Average, 1-12.....	9.2	9.4	10.0	9.0	9.4	.214	.....

EXPOSED SUBSOIL

1.....	12.7	12.5	.....	.....	12.6	.047	.....
2.....	12.7	12.6	.....	.....	12.6	.047	.....
3.....	12.8	13.0	.....	.....	12.9	.045	0.25
4.....	13.2	13.2	.....	.....	13.2	.044	.....
5.....	13.2	12.6	.....	.....	12.9	.044	.....
6.....	13.2	12.5	.....	.....	12.9	.041	.....
7.....	12.8	12.6	.....	.....	12.7	.040	.....
8.....	12.7	12.6	.....	.....	12.7	.038	.18
9.....	12.7	12.7	.....	.....	12.7	.038	.....
10.....	12.3	12.4	.....	.....	12.4	.038	.....
11.....	12.4	12.7	.....	.....	12.6	.038	.....
12.....	12.2	12.9	.....	.....	12.6	.036	.18
Average, 1-6.....	13.0	12.7	.....	.....	12.9	.....	.....
Average, 7-12.....	12.5	12.6	.....	.....	12.6	.....	.....
Average, 1-12.....	12.7	12.7	.....	.....	12.7	.041	.....

Four sets of samples from this fallow area and two sets from the exposed subsoil, all taken for moisture determinations, were employed for the determination of the hygroscopic coefficient. The data from the different sets from the same field (Table XXII) are so similar for the corresponding soil layers of the surface foot that it appears permissible



to use the average values for all of the 12 inches in calculating the ratios. The nitrogen and organic carbon content reported in the same table show the typical character of the one as a surface soil and of the other as a subsoil. The average hygroscopic coefficient for the surface foot from the fallow field was 9.4 compared with 10.2 for the surface soil D, while that for the surface foot of the exposed subsoil is 12.7 compared with 13.3 for the subsoil A.

A Government rain gauge, maintained at the Experiment Station and within 400 yards of both sampled areas, furnished the data on the rainfall. The weather of the four months involved did not depart widely from the normal at Lincoln, except that the May rainfall, amounting to 0.69 inch occurring in five showers (0.10 inch on the 1st, 0.32 on the 4th, 0.15 on the 10th, 0.02 on the 20th and 0.10 on the 26th) was 3.64 inches below the normal. The rainfall for June, July, and August 1 to 26 amounted to 4.03, 2.68, and 3.86 inches, respectively. Both areas were sufficiently far from trees and alfalfa plants to avoid any draft by roots upon the subsoil moisture.

The compact, uncultivated, but weedless, and gently sloping surface of the exposed subsoil was unfavorable to the ready penetration of the rains and favorable to run-off, while the loose, almost level surface of the fallow field permitted ready penetration and prevented any serious loss by run-off.

The ratios for the surface inch and the four 3-inch sections are reported in Table XXIII. While on both areas the ratio in the surface 3-inch section fell very low during dry weather, at depths below this in the exposed subsoil it remained very constant, varying only from 1.9 to 2.4; but in the fallow it ranged from 2.1 to 3.9, the former after dry weather and the latter very soon after a heavy rain. In the fallow field the lowest 3-inch section showed a lower ratio than the overlying two sections, in this resembling the exposed subsoil.

TABLE XXIII.—Ratio of water content to hygroscopic coefficient at different levels in the surface foot of two adjacent fields

Date.	Weather conditions.	Bare subsoil.					Fallow field.				
		First inch.	1 to 3 inches.	4 to 6 inches.	7 to 9 inches.	10 to 12 inches.	First inch.	1 to 3 inches.	4 to 6 inches.	7 to 9 inches.	10 to 12 inches.
May 25-27.....	Prolonged hot dry weather followed by 0.10 inch rain.....	0.8	1.0	2.0	2.1	2.0	0.5	1.1	2.8	2.9	2.4
June 1.....	Within 8 hours after 0.50 inch rain.....	2.4	2.1	2.0	2.2	2.2	2.6	2.0	2.9	2.9	2.4
June 14.....	Within 5 hours after 2.71 inches rain.....	2.3	2.3	2.3	2.3	2.4	3.9	3.9	3.7	3.6	3.0
July 10.....	Prolonged hot dry weather followed by 0.24 inch rain.....	1.7	1.6	1.9	2.0	2.0	1.8	1.5	2.8	3.2	2.5
July 11.....	Within 4 hours after 0.81 inch rain.....	1.9	1.7	1.9	2.1	2.3	3.4	3.2	3.1	3.1	2.5
Aug. 3.....	After a week of dry weather.....	.9	1.3	2.0	2.1	2.2	2.7	2.5	2.1	3.1	2.5
Aug. 6.....	Within 6 hours after 1.30 inches rain.....	1.8	1.8	1.9	2.1	2.2	3.6	3.5	3.4	3.1	2.5
Aug. 16.....	Within 8 hours after 2.11 inches rain.....	2.1	2.1	2.1	2.2	2.1	4.0	4.0	3.9	3.4	2.7
Aug. 26.....	After 10 days of dry weather.....	1.2	1.7	2.0	2.0	2.1	1.7	2.3	3.1	3.0	2.5

Data on the first 5 feet of soil from the same two fields are available (Table XXIV). These are from samplings on June 19 and August 29, both sets of samples being composites from three borings. If we omit the first foot, the ratios for the different levels below the bare subsoil vary from 2.2 to 2.6, while with the fallow field they lie between 1.2 and 1.9. The lower ratios in the latter are clearly to be attributed, not to a lesser ability to retain moisture against seepage, but to the precipitation's not having been sufficient to raise the moisture content of the subsoil of the fallow to its upper limit after its having been reduced to a very low point by the crop on the field in 1911.

TABLE XXIV.—Ratio of water content to hygroscopic coefficient at lower levels in the two fields mentioned in Table XXIII

BARE SUBSOIL					
	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.
Hygroscopic coefficient.....	12.2	12.2	12.2	11.8	12.0
Total water, June 19.....per cent..	26.9	28.9	29.6	29.1	31.2
Total water, August 29.....do.....	24.8	26.8	27.4	28.7	29.2
Ratio, June 19.....	2.2	2.4	2.4	2.5	2.6
Ratio, August 29.....	2.0	2.2	2.2	2.4	2.4

FALLOW					
	10.6	14.7	13.5	13.0	13.0
Hygroscopic coefficient.....	10.6	14.7	13.5	13.0	13.0
Total water, June 19.....per cent..	28.1	17.8	21.7	24.2	24.5
Total water, August 29.....do.....	23.9	27.2	23.8	22.1	23.5
Ratio, June 19.....	2.6	1.2	1.6	1.9	1.9
Ratio, August 29.....	2.3	1.8	1.8	1.7	1.8

In the case of the two fields just mentioned conditions were not such as to induce the maximum downward movement of the water contained in the surface foot, the underlying layers having a moisture content far above the hygroscopic coefficient. However, the data we obtained near McCook, Nebr., during the same summer, that of 1912, are strictly comparable with those obtained in the 2-foot cylinders, the moisture content of the underlying subsoil approximating the hygroscopic coefficient.

McCook, which is in the semiarid portion of Nebraska, had experienced a series of remarkably dry years. Between the middle of August, 1910, and the first of the following August there were only 6.36 inches of precipitation. During August there fell 4.34 inches, but this was followed by dry weather, so that by the advent of frost the moisture of the soil and subsoil had been reduced to approximately the hygroscopic coefficient. Two rains in the following March and two in

April, one of 0.55 inch on the 20th and the other of 1.38 on the 28th, moistened the surface. About a week after the latter date, on May 6 to 8, while the surface 6-inch section was still moist from the recent rain, samples to a depth of 6 feet were taken from nine fields near McCook. The hygroscopic coefficients and the ratios are reported in Table XXV. In the case of three prairie fields in which the moisture content of the subsoil had been reduced the previous season to practically the hygroscopic coefficient, the rains had raised the ratio of the whole of the first foot to 2.1 to 2.3, the upper half being the more moist; the second foot also had been affected, the ratio in it having been raised to 1.3 or 1.4. One field of wheat (winter) and one field that had borne corn the previous year and on which the stubble was still undisturbed showed similar moisture conditions; while the two other wheat fields and the remaining two with corn stubble, although showing similar moisture conditions on the surface, exhibit a higher ratio in the subsoil, as though the previous year's crops had not fully exhausted this of its moisture. If we compare the nine fields, we find the ratio in the surface foot to vary between 1.8 and 2.8.

TABLE XXV.—Ratio of moisture content to hygroscopic coefficient in fields near McCook, Nebr., on May 6, 7, and 8, 1912

Depth.	HYGROSCOPIC COEFFICIENT								
	Prairie fields.			Wheat fields.			Corn stubble.		
	I.	II.	III.	I.	II.	III.	I.	II.	III.
<i>Feet.</i>									
0 to ½.....	8.6	8.0	8.5	7.3	8.2	8.5	10.8	8.5	8.6
½ to 1.....	10.6	10.9	11.7	8.6	10.3	10.7	11.4	10.1	10.3
2.....	10.3	9.6	10.1	7.8	9.8	9.2	9.7	9.1	11.9
3.....	8.3	8.4	.....	9.4	8.4	8.6	8.6	8.5	9.0
4.....	7.5	8.7	.....	8.7	7.8	9.0	8.0	8.1	8.3
5.....	7.8	8.4	7.9	8.6	7.5	8.8	8.0	7.7	8.3
6.....	7.6	7.2	.....	7.5	7.4	7.0	7.9	8.5	8.0

RATIO OF MOISTURE CONTENT TO HYGROSCOPIC COEFFICIENT									
0 to ½.....	2.3	2.5	2.3	2.8	2.5	2.1	1.8	1.8	2.2
½ to 1.....	2.0	2.2	2.0	2.6	2.1	2.0	1.9	1.9	2.1
2.....	1.4	1.4	1.3	2.0	1.6	1.2	1.4	2.1	1.5
3.....	1.1	1.0	(a)	1.1	1.5	1.3	1.0	1.6	1.2
4.....	1.0	1.0	(a)	1.1	1.4	1.3	1.1	1.2	1.2
5.....	1.0	1.1	1.0	1.1	1.3	1.1	1.1	1.2	1.1
6.....	1.1	1.3	(a)	1.3	1.2	1.2	1.1	1.1	1.2

<sup>a</sup> Samples lost.

No rain fell between April 28 and June 6; but between the latter date and June 18 there were nine rainy days, with a total precipitation of 2.77

inches. On June 26 and 27 we made an exhaustive study of the moisture conditions in a level field about 3 miles from the W. B. station. It had been plowed the previous autumn and had since been kept free of weeds, being planted to corn about June 1. At the time of our sampling, the corn plants, 8 to 10 inches high, in hills 3 feet 8 inches apart, were still too small to have made any considerable draft upon the soil moisture. Selecting a level portion of the field, one which would not be affected by run-off from higher land, or itself lose much water by run-off, we marked 25 sites for sampling, 10 yards apart from north to south and the same from east to west (fig. 3). Sites 1, 5, 21, and 25 were at the four corners and 13 at the center. These five were sampled to a depth of 6 feet with an auger. All the others were sampled to a depth

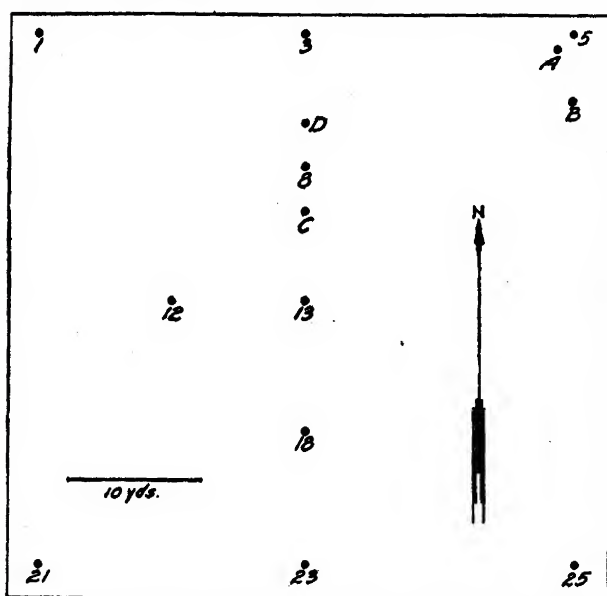


FIG. 3.—Diagram showing the relative location of sets of soil samples taken from field near McCook, Nebr.

of 4 feet, a tube being used on part of them. The moisture content and the hygroscopic coefficient were determined in the case of each member of the 25 sets. The moisture conditions were very similar in all. The moisture content of the second foot had been distinctly affected at each place, while in five (including Borings I and V in Table XXVI) that of more or less of the third foot also had been affected; but in none had that of the fourth foot been appreciably influenced. The data on the five 6-foot borings are given in Table XXVI. The ratio in the first two lies between 2.0 and 2.9, while in the fourth and fifth feet it is approximately 1.1. From a comparison of this table with the preceding it will be seen that in the seven weeks following the earlier sampling the moistened zone in the fields in clean cultivation appears to have extended downward about 1 foot.

To secure information as to how abrupt was the change in moisture content within a foot section in the case of seven borings, we separated into two parts the foot section that included the transition from moist to very dry soil. The upper section was the more or less moist portion,

possessing enough coherence to be removed from the boring by an ordinary soil auger, while the lower was the portion that was too dry and powdery to adhere to the ordinary auger and which had to be removed by a special auger carrying a sleeve. The data are reported in Table XXVII.

TABLE XXVI.—Ratio of moisture content to hygroscopic coefficient in a clean cornfield near McCook, Nebr., on June 26 and 27, 1912

HYGROSCOPIC COEFFICIENT						
Depth.	Individual borings No.					
	I.	II.	III.	IV.	V.	Average.
<i>Feet.</i>						
1.....	8.1	9.3	7.7	8.1	9.7	8.6
2.....	9.2	9.2	8.0	10.5	10.3	9.4
3.....	10.0	8.0	8.3	10.1	8.9	9.0
4.....	8.1	7.5	8.0	8.8	8.6	8.2
5.....	8.2	7.6	7.3	8.1	7.9	7.8
6.....	8.3	7.5	6.7	8.1	7.6	7.8
RATIO OF MOISTURE CONTENT TO HYGROSCOPIC COEFFICIENT						
1.....	2.6	2.6	2.9	2.7	2.4	2.6
2.....	2.2	2.1	2.0	2.1	1.8	2.0
3.....	1.3	1.1	1.0	1.0	1.6	1.2
4.....	1.1	1.2	1.0	1.0	1.1	1.1
5.....	1.1	1.1	1.0	1.1	1.1	1.1
6.....	1.2	1.2	1.4	1.1	1.2	1.2

TABLE XXVII.—Comparison of the moisture conditions in the upper and lower part of the foot section that formed the transition from moist to very dry soil

Boring No.	Depth.	Field notes.	Moisture.	Hygroscopic coefficient.	Ratio.
	<i>Inches.</i>		<i>Per cent.</i>		
1.....	25-28	Moist.....	17.0	10.0	1.7
	29-36	Dry.....	11.2	10.0	1.1
3.....	13-14	Moist.....	20.5	11.4	1.8
	15-24	Dry.....	15.1	10.0	1.5
8.....	13-21	Moist.....	20.3	11.5	1.8
	22-24	Dry.....	12.4	10.4	1.2
12.....	13-22	Moist.....	21.2	11.2	1.9
	23-24	Dry.....	14.7	<sup>a</sup> 11.2	1.3
18.....	13-21	Moist.....	18.7	10.7	1.7
	22-24	Dry.....	12.8	<sup>a</sup> 10.7	1.2
23.....	13-22	Moist.....	19.7	10.9	1.8
	23-24	Dry.....	12.8	<sup>a</sup> 10.9	1.2
25.....	13-18	Moist.....	15.9	8.9	1.8
	19-24	Dry.....	13.5	<sup>a</sup> 8.9	1.5

<sup>a</sup> The two portions of the foot section had been combined before the hygroscopic coefficient was determined.

The ratio in the moist upper portion of the foot section was 1.7, 1.8, or 1.9, while that in the dry "powdery" portion was distinctly lower—



1.1 to 1.5. The change in ratio here is even more abrupt than in the cylinders which had been allowed to reach equilibrium (Table XV).

In order to study the transition from moist to very dry soil in greater detail, sets of samples were taken with the inch sampler (previously described) in four different places in the same areas (fig. 3). In each set the sampled depth, including the whole of the moistened zone, extended from the surface well into the underlying dry zone. As will be seen from Table XXVIII, the samples were taken in such short sections as to make the results strictly comparable with those obtained with the cylinders. The ratio was found to fall within a short distance, 5 to 11 inches, from 2.0 to 1.1,—that is to say, from a condition not far from the optimum moisture content to one too dry to permit root development, and in which the soil is almost completely exhausted of available water, so far as ordinary crop plants are concerned. In set D, in which the transition was most gradual, this change required 16 inches. In each set the maximum ratio lay between 2.1 and 2.4, the maximum being somewhat lower than found in the first foot sections reported in Table XXVI.

TABLE XXVIII.—Ratio of moisture content to hygroscopic coefficient at different distances from the surface in a clean cornfield near McCook, Nebr., on June 26 and 27, 1912

Set A.			Set B.			Set C.			Set D.		
Depth of section.	Hygroscopic coefficient.	Ratio.	Depth of section.	Hygroscopic coefficient.	Ratio.	Depth of section.	Hygroscopic coefficient.	Ratio.	Depth of section.	Hygroscopic coefficient.	Ratio.
Inches.			Inches.			Inches.			Inches.		
1.....	8.0	0.4	1.....	7.8	0.4	1.....	7.4	0.7	1-3.....	8.8	1.9
2.....	8.3	1.9	2.....	8.3	1.4	2.....	8.0	1.8	4-6.....	11.3	2.1
3.....	9.3	2.3	3-4.....	9.8	2.4	3-4.....	8.7	2.4	7-9.....	11.6	2.1
4.....			5-6.....	10.7	2.2	5-6.....	9.8	2.3	10-12.....	11.5	1.8
5-6.....	9.5	2.3	7-8.....	11.5	2.1	7-8.....	10.5	2.2	13.....	10.7	1.8
7-8.....	10.9	2.0	9-10.....	11.4	2.1	9-10.....	12.2	1.9	14.....	10.4	1.8
9-10.....	12.5	1.9	11-12.....	11.6	1.9	11-12.....	11.1	2.0	15.....	10.4	1.8
11-12.....	11.7	2.0	13.....	11.1	1.9	13.....	11.1	1.9	16.....		
13-14.....	12.1	1.7	14.....	10.4	2.0	14.....	10.6	2.0	17.....	10.8	1.7
15-16.....	11.2	1.7	15.....	10.4	1.9	15.....	10.5	1.8	18.....	10.7	1.6
17-18.....	10.2	1.6	16.....	9.4	2.0	16.....	10.6	1.7	19.....	9.8	1.8
19-20.....	9.9	1.1	17.....	9.4	2.0	17.....	10.5	1.5	20.....	10.3	1.6
21-22.....	9.9	1.0	18.....	9.1	2.0	18.....	10.5	1.2	21.....	10.3	1.5
23-24.....	9.6	1.0	19.....	9.2	2.0	19.....	10.6	1.1	22.....	9.7	1.5
25-26.....	9.3	1.0	20.....	9.2	2.0	20.....	10.6	1.0	23.....	9.7	1.4
27-28.....	9.1	1.0	21.....	9.1	2.0	21.....	10.6	.9	24.....	9.8	1.3
29-30.....	8.7	1.0	22.....	9.4	1.8	22.....	10.8	.9	25.....	9.4	1.2
31-32.....	8.1	1.0	23.....	9.2	1.9	23.....	10.1	.9	26.....	9.7	1.1
33-34.....	8.2	1.0	24.....	9.0	1.8	24.....	9.7	1.0	27.....	9.6	1.0
35-36.....	8.6	1.0	25.....	8.9	1.8	25.....			28.....		
			26.....	9.0	1.6	26.....	9.7	.9	29.....	9.0	1.0
			27-28.....	9.0	1.3	27-28.....	9.7	1.0			
			29-30.....	8.6	1.1	29-30.....	9.3	.9			
			31-32.....	8.0	1.1	31-32.....	8.5	1.0			
			33-34.....	8.0	1.1	33-34.....	8.4	1.0			
			35-36.....	8.3	1.1	35-36.....	8.2	1.0			

C.—FINE SANDS.—Our field data on soils with hygroscopic coefficients between 1.1 and 3.0 such as fine sands, are very scanty and only where the samples were taken under conditions permitting an accumulation of moisture in the subsoil are they of interest in the present connection.



Under such conditions these soils show ratios between 3 and 5 (Table XXIX) and thus are intermediate between the coarse sands and the loams.

TABLE XXIX.—Maximum ratios of water content to hygroscopic coefficient in Nebraska sandy soils

HYGROSCOPIC COEFFICIENT							
Depth.	Near Madrid.		Near Valentine.		Near Imperial.		
	Prairie, May 1, 1908.	Prairie, Mar. 25, 1910.	Corn, Aug. 21, 1911.	Prairie, Aug. 21, 1911.	Corn, stubble, May 11, 1912.	Prairie, May 11, 1912.	Prairie, May 11, 1912.
Feet.							
1.....	1.9	1.9	3.3	1.4	2.5	2.6	1.6
2.....	1.8	1.7	2.2	1.4	2.6	3.7	1.6
3.....	1.7	1.7	1.4	1.4	2.6	3.5	1.9
4.....	1.5	1.4	.9	1.2	3.0	3.5	1.5
5.....	1.8	1.4	.9		5.6	1.6	1.3
6.....	1.9	1.5	1.0		8.9	1.3	1.3

RATIO							
1.....	2.6	2.6	2.2	4.1	3.4	2.8	2.5
2.....	3.1	3.6	1.2	4.1	3.3	2.8	2.1
3.....	2.9	3.4	1.6	2.3	3.3	2.7	4.2
4.....	2.4	4.7	3.5	3.9	3.3	2.3	4.6
5.....	3.7	4.5	3.9		2.8	3.1	4.5
6.....	3.1	4.6	3.7		2.4	3.4	4.7

A later experiment in which several soils intermediate in texture were used along with those mentioned in Table I throws some light on the

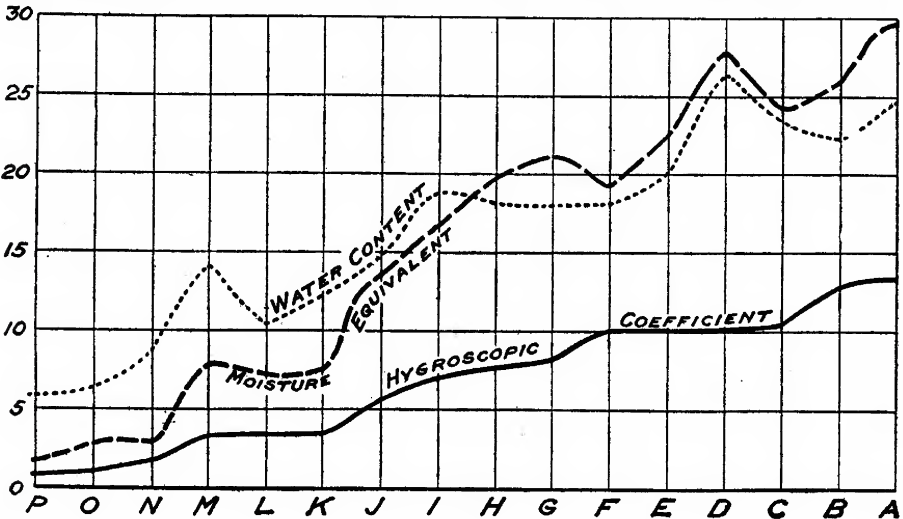


FIG. 4.—Diagram showing relation of water content of the surface section to both the hygroscopic coefficient and the moisture equivalent five days after 1 inch of water had been added to the surface of columns of dry soils.

conduct of these (Table XXX and figure 4). To the surface of columns of the soils containing an amount of water approximately half the hygroscopic

coefficient there was added 1 inch of water. The cylinders were allowed to stand protected from evaporation for five days, and then the moisture content of the upper 2 or 3 inches of moistened soil determined.

TABLE XXX.—Ratio of moisture content to hygroscopic coefficient in the surface section five days after 1 inch of water had been added to the surface of the column of dry soil

Soil No.	Hygroscopic coefficient.	Initial moisture content.	Moisture content.	Ratio.
		<i>Per cent.</i>	<i>Per cent.</i>	
A.....	13.3	6.6	24.6	1.9
B.....	12.9	6.3	22.2	1.7
C.....	10.5	5.0	23.3	2.2
D.....	10.2	4.9	26.4	2.6
E.....	10.1	5.0	20.1	2.0
F.....	10.0	4.8	18.0	1.8
G.....	8.2	3.9	18.0	2.2
H.....	7.6	3.8	18.0	2.4
I.....	7.1	3.5	18.8	2.6
J.....	5.6	2.8	14.6	2.6
K.....	3.4	1.7	12.3	3.6
L.....	3.4	1.6	10.5	3.1
M.....	3.3	1.7	14.1	4.3
N.....	1.7	.6	8.8	5.2
O.....	1.1	.4	6.4	6.6
P.....	.9	.2	5.9	6.6
Q.....	.6	.2	9.0	15.0

The retardation of movement appears to increase with the decrease in the coefficient when this is below 2.0; and even with K, L, and M, with coefficients between 3.0 and 4.0, a retardation is apparent during the first five days following the application of water, although, as shown above, this was not observable after the lapse of two months.

#### RÔLE OF THE MOISTURE OF THE DEEP SUBSOIL

From the above statements it would appear that a definite answer may be given to the question as to how far the water in the deeper subsoil is of importance to annual crop plants. As the minimum to which crops can reduce the moisture in the upper subsoil—that traversed by the roots—is approximately 1.0 to 1.1 times the hygroscopic coefficient; and, as the maximum to be expected in the deeper subsoil is 1.7 to 2.5 in the case of loams, it becomes a question of how far and how rapidly the moisture in a layer in which the ratio is 2.5 will move upward into an overlying layer with a lower ratio of, say, 1.0 to 1.1. The experiments have shown that equilibrium may be practically attained when these extremes are to be found as close together as 2 feet, or even less, and that the movement is so limited that three months was insufficient to restore, by upward capillary movement, the small amount of moisture lost during 2½ days into the still air of a basement room from the surface of soils with a ratio of 1.0. While a very much higher ratio is encountered

in coarse sands when the downward movement has become so slight as to be almost negligible, the upward movement in these is limited to very short distances. From these considerations it is evident that the amount of water which the deep subsoil can contribute to the growth of annual-crop plants will be of no practical importance. When a perennial crop with a root range of 20 to 30 feet follows an annual with one of only 4 to 6 feet, the moisture of the deeper subsoil becomes of great importance; but here it is a case of the roots going to the moisture and not of this being elevated to them by capillarity.

The experiments described, however, do not answer the question as to whether the moisture of the deeper subsoil may not, in the course of several years, or of a few decades, be elevated through a much greater distance. For instance, whether after the subsoil to a depth of 20 or 30 feet has been exhausted of available moisture (brought to a ratio of 1.0 to 1.1) by deep-rooted perennials, it may not eventually have the ratio restored to 1.7 to 2.4 by capillary movement from the deeper subsoil instead of only by the portion of the precipitation reaching it from above. Even if a decade were required for such a transfer, the elevated moisture might still have some practical importance for the perennial crops. Field investigations and laboratory experiments which would definitely decide this point appear simple in principle. The character of suitable cylinder experiments will be evident from those discussed above. For these the calcareous loessial silt loams of the Great Plains, and the so-called "volcanic ash" of eastern Washington and Oregon would be especially suitable.

A field study, if sufficiently thorough, would give a more satisfactory answer, but it would be far more laborious, and in most places would appear to be quite impracticable. It should necessarily be conducted in a region of limited rainfall, and even there a wet year or two might cause the experiment to miscarry entirely. Desirable conditions would include a silt-loam subsoil comparatively uniform to a depth of 40 feet, free of any interrupting sand or gravel layers, and a water table at a depth of not less than 100 feet. The subsoil of the field practically exhausted of available moisture to a depth of 20 or 30 feet, as by a long continued stand of alfalfa, should be sampled at intervals of a foot or so from the surface through the dry zone and well into the moist deeper subsoil. The places of sampling should be sufficiently numerous to establish the uniformity of the distribution of moisture; and with all samples there should be a determination of the hygroscopic coefficient or moisture equivalent, as well as of the total moisture. In beginning the study the perennial crop on the experimental field should be at once killed to prevent further loss by transpiration, and only annuals should be allowed on it during the experiment. The thickness of stand and the period of growth of these annuals should be such as to intercept most

completely the precipitation. The deep samplings for moisture determinations should be sufficiently numerous, frequent, and deep to afford a reliable history of the moisture content in the moist lower subsoil, in the central dry zone, and in the intermittently moistened surface layer. To illustrate the difficulty of securing a satisfactory site for such a field study, it might be mentioned that in no part of Minnesota would it appear feasible, either the climate being too humid, the water table too near the surface, or, lastly, the subsoil being too shallow where free of rock fragments and, where deep enough, carrying too many such fragments to permit satisfactory sampling.

#### SUMMARY

Uniform columns of soil of known hygroscopic coefficient and moisture equivalent were employed in various laboratory experiments, the 13 soils used ranging in texture from a coarse sand to a silt loam with hygroscopic coefficients of 0.6 and 13.3, respectively.

Five of the loams, placed in capillary connection with the natural subsoil mass, saturated with water and allowed to stand protected from surface evaporation for several months, lost water until the amount retained bore a close relation to the hygroscopic coefficient, being from 2.1 to 3.1 times this value, according to the particular soil. When a layer of coarse sand or gravel separated the column of loam from the natural subsoil mass or interrupted it, the downward movement of the water in the soil above this layer was much delayed. Where the column consisted of successive 2-inch layers of loams differing widely in texture, the order of their arrangement exerted no influence upon their final water content.

Soil columns 30 to 36 inches long, while protected from all loss of moisture at the sides and bottom, were freely exposed to evaporation at the surface for periods varying from a few weeks to half a year. The moisture content, originally uniform and lying between 2.0 and 3.0 times the hygroscopic coefficient, fell until it reached, at depths below the first foot, an almost constant minimum with the ratio 1.9 to 2.2.

Employing 2-foot columns of 12 different loams, each with an initial moisture content approximately equal to its hygroscopic coefficient, enough water was added to raise the average moisture content of the column to 1.5 times the hygroscopic coefficient, the water being applied in one experiment to the top and in another to the base of the column. After the cylinders had stood for three or four months fully protected from evaporation the distribution of moisture, with regard to the surface to which it had been applied, was found to be the same in both experiments. The maximum distance through which an effect was shown was about 2 feet, but in most cases much less. The maximum final ratio of moisture content to hygroscopic coefficient was found in

the section adjacent to the surface of application, where it lay between 1.7 and 2.4. The ratio, while falling within these limits, is not a constant, it not being the same for all the soils that have the same hygroscopic coefficient.

The water-retaining capacity of the loams, as determined by laboratory experiments, was found to bear a somewhat closer relation to the moisture equivalent than to the hygroscopic coefficient, the ratio varying between 0.8 and 1.2.

Coarse sands exhibited a behavior very different from that of the loams. The ratio in the surface 6-inch section, even three months after 1 inch of water had been applied to the surface, was as high as 6.0 or 7.0, while in the second foot it was only 1.0. The field studies on coarse sands showed as high a final ratio as was observed in the laboratory experiments.

The very limited studies on fine sands indicate that these occupy a position intermediate between the loams and the coarse sands, the ratio of the water-retaining capacity to the hygroscopic coefficient rising as the latter value falls.

Field studies show that when loams, after rains sufficiently heavy to moisten them thoroughly, are protected from losses by evaporation and transpiration, they lose water by downward movement until the ratio of moisture content to hygroscopic coefficient lies between 1.8 and about 2.5, and accordingly on the uplands of dry-land regions this is the ratio to be expected in the deeper subsoil—the portion below the range of plant roots.

A comparatively abrupt transition from the moistened soil to the thoroughly exhausted underlying layers, with ratios of 2 to 2.5 and 1.0 to 1.1, respectively, is found even several months after liberal rains have fallen, if the subsoil to a considerable depth had previously been exhausted of available water.

The moisture of the deeper subsoil will be able to move upward only so slowly and through such a short distance in a single season that it will be at most of no practical benefit to annual crops. To make use of any portion of the precipitation which penetrates beyond the reach of the roots of annual crops it will be necessary to follow such crops at intervals by deep-rooting perennials.

Further experiments of a long-time character are necessary to decide definitely whether the deep subsoil may not in a decade or so contribute sufficient moisture to the subsoil within the reach of the roots of such perennials, 20 to 30 feet, to make such a contribution of some practical importance for such crops.



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## ABSORPTION OF NUTRIENTS AS AFFECTED BY THE NUMBER OF ROOTS SUPPLIED WITH THE NUTRIENT

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### INTRODUCTION

In the course of several investigations on the mineral nutrition of rice (*Oryza sativa*) it became necessary to know whether the plant could absorb an optimum amount of the mineral element which was supplied to only part of the roots if all the other essential elements were supplied to all the roots. At first thought it would seem the plant could absorb sufficient of the element supplied to only part of its roots if sufficient selection could be exercised in the absorption of the different nutrients by individual roots. So far as known, no quantitative study has been made of this point; hence, the tests reported below were conducted.

The selective absorption of mineral elements was early established by Saussure,<sup>1</sup> Gorup-Besanez,<sup>1</sup> and W. Wolf (12).<sup>2</sup> E. Wolff (13) showed that the selective absorption could be altered by changing the proportion of salts in the nutrient solution. Some recent studies on antagonism have also demonstrated that absorption or penetration of ions in plant roots is affected by the relative concentrations of ions in the solution. Most of such studies, however, have been made on unbalanced solutions of one or two salts only, and the object has been to ascertain the influence of different proportions of salts on root growth aside from nutritive effects. The results show that a nutrient solution, to be a proper medium for root growth, must contain the salts in certain proportions. These proportions may be varied considerably, especially in low concentrations of a variety of salts, without appreciably affecting growth (3, 11).

The present work does not deal with the effect of the medium on selective absorption by roots, but with the effect of localization of the supply. The results emphasize to what a great extent selective absorption may be altered by limiting the supply of an element to a few roots, without changing the medium. Some work done previously is similar to this investigation, in that plants were grown with their roots divided between two unlike media. Quantitative data, however, were not secured on the point which is the subject of this work.

<sup>1</sup> Cited by Heiden (6, p. 280-286). <sup>2</sup> Reference is made by number to "Literature cited," p. 94-95.

Frank (2, p. 153) grew corn and peas with roots divided between two compartments, one of which contained calcium nitrate and the other of which did not. The only result reported was that roots in the compartment with nitrate made a much more luxuriant development of side roots. He describes the experiment as being similar to one of Müller-Thurgau's. Nobbe (9), growing corn in pots with fertilizer applied to different parts of the soil, also observed that the development of lateral roots was much greater in those zones where fertilizers had been applied.

Faack (1) grew wheat with part of the roots in a solution lacking one element and a few roots in a solution containing the single salt not present in the main solution. He found that the plant would grow without marked disturbance under these conditions if the proper salt and proper concentration were used in the single salt solution. As only two plants per lot were used, his data do not show the extent to which growth and absorption were affected.

#### METHOD OF EXPERIMENTS

The experiments were carried out in water cultures, as it is obviously difficult to maintain a good separation of roots and localize the distribution of salts in sand cultures. Erlenmeyer flasks of "Nonsol" or Jena glass joined together at the necks were used as receptacles. The plants were grown with their roots divided between the two flasks, one of which contained a complete nutrient solution and the other a nutrient solution lacking one element.

Rice seedlings were germinated over distilled water for Experiments I, III, and IV, but in tap water for subsequent experiments, as root development was better in tap water and there was no need of excluding all mineral salts before starting the experiment. Corn (*Zea mays*) was germinated in sphagnum moss. One corn seedling or two rice seedlings were grown in each double flask.

The compositions of the nutrient solutions used are shown in Table I.

TABLE I.—*Composition of nutrient solutions used*

Solution.	Complete acid solution.	Complete neutral solution.	Nitrogen-free solution.	Phosphorus-free solution.	Potassium-free solution.
	Gm.	Gm.	Gm.	Gm.	Gm.
Potassium nitrate ( $\text{KNO}_3$ ).....	10.71	10.71	.....	10.71	.....
Monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ )....	7.14	3.57	3.57	.....	.....
Dipotassium phosphate ( $\text{K}_2\text{HPO}_4$ ).....	.....	3.57	3.57	.....	.....
Monosodium phosphate ( $\text{NaH}_2\text{PO}_4$ ).....	.....	.....	.....	.....	6.30
Sodium nitrate ( $\text{NaNO}_3$ ).....	21.43	21.43	.....	21.43	14.30
Sodium sulphate ( $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ ).....	3.15	3.15	3.15	.....	3.15
Calcium chlorid ( $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ ).....	2.00	2.00	37.60	2.00	.....
Magnesium chlorid ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ).....	2.00	2.00	35.60	2.00	2.00
Potassium sulphate ( $\text{K}_2\text{SO}_4$ ).....	.....	.....	12.40	6.20	.....
Calcium nitrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ).....	.....	.....	.....	.....	21.86
Iron (Fe).....	.80	.80	.80	.80	.80
Sulphuric acid ( $\text{H}_2\text{SO}_4$ ).....	.25	.....	.....	.....	.25
Distilled water.....	.....	.....	100,000 c. c.	.....	.....

The form in which the iron was added varied in some experiments, although it was used at the rate of 0.8 gm. of iron per 100,000 c. c. in all experiments except IX. The iron-free solution was the same as the complete solution, except for the absence of iron. Water used in the nutrient solutions was distilled from a cast-iron still with tin condenser and stored in a tin-lined copper tank.

The solutions were generally changed every four days, except that, when the plants were first inserted, they were left for six days. Sometimes when growth was especially rapid, as with corn, the solutions were renewed more frequently. Transpired water was made up with distilled water every day. In all the experiments rice was grown for 40 days and corn for 21 days.

In analyzing the plants the usual analytical methods were used, except that iron was determined by the colorimetric method with sulphocyanic acid (HSCN) (10).

EXPERIMENTAL RESULTS

EXPERIMENT I.—RICE WITH HALF THE ROOTS IN A NITROGEN-FREE SOLUTION

In this test the complete neutral and nitrogen-free solutions shown in Table I were used, with ferrous sulphate as the source of iron. In one lot half the roots were maintained in the complete and half in the nitrogen-free solution. In other lots all the roots were kept either in the complete solution or in the nitrogen-free solution. The plants without nitrogen were grown in 200-c. c. flasks for 40 days; all others in 200-c. c. flasks for 22 days, and in 500-c. c. flasks for 18 days. Six double flasks were taken as a unit and the units triplicated for each treatment. Solutions were changed seven times in the course of the experiment. Experimental data are given in Tables II and III.

TABLE II.—Growth of rice in Experiment I

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
			Gm.	Gm.	Gm.			Gm.	Gm.	
Nitrogen-free.....	Nitrogen-free.....	1-6	1.69	0.43	0.42	49	51	0.119	0.144	0.626
		7-12	1.62	.41		44	47			
		13-18	1.71	.42		41	58			
		19-24	78.46	11.73		195	195			
Do.....	Complete.....	25-30	80.83	11.88	11.29	231	188	2.271	2.274	.403
		31-36	73.54	10.25		187	190			
		37-42	96.77	13.13		215	193			
		43-48	99.44	13.31		225	194			
Complete.....	.....do.....	49-54	86.12	12.36	12.93	196	204	2.198	2.044	.328



TABLE III.—Nitrogen and phosphoric acid absorbed by rice in Experiment I

Flask No.	Nitrogen (N) in dry stalks and leaves.	Nitrogen (N) in dry roots.		Nitrogen (N) absorbed by 12 plants.	Nitrogen (N) absorbed per gram of roots.	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) in dry stalks and leaves.
		A-flasks.	B-flasks.			
	Per cent.	Per cent.	Per cent.	Gm.	Gm.	Per cent.
1-18	1. 32	0. 97	0. 97	.....	.....	.....
19-36	3. 90	1. 47	2. 12	0. 5138	0. 226	1. 61
49-54	4. 40	2. 41	2. 41	. 6631	. 156	1. 54

If the plants with all their roots in the complete solution are considered as normal in growth and nitrogen content, it is evident that the plants partially supplied with nitrogen made slightly less than normal growth, had 23 per cent greater ratio of roots to tops,<sup>1</sup> and absorbed 0.77 of the normal amount of nitrogen. The influence of the need of the plant on the selective absorption of nitrogen is shown by the amount of nitrogen absorbed per gram of roots, the partial-nitrogen plants having absorbed 1.45 times as much nitrogen as the normal plants.

#### EXPERIMENT II.—CORN WITH HALF THE ROOTS IN NITROGEN-FREE SOLUTION

It was thought desirable to repeat the preceding test with corn, so that the results might not be taken as being peculiar to rice alone. Because of the rapid growth, corn could be grown for 21 days only instead of 40, with the means at our disposal.

No-nitrogen plants were grown in 200-c. c. flasks for the 21 days; all others in 200-c. c. flasks for 11 days, 500-c. c. flasks for 4 days, and 1,000-c. c. flasks for 6 days. The nutrient solutions used were the same as in Experiment I, and were changed six times during the experiment. Eight flasks were taken as a unit and the units duplicated for each treatment. Experimental data are given in Tables IV and V.

TABLE IV.—Growth of corn in Experiment II

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
			Gm.	Gm.	Gm.			Gm.	Gm.	
Nitrogen-free.....	Nitrogen-free.....	{ 1-8	12.4	1.55	{ 1.91	{ 37	46	{ 0.80	{ 0.71	0.791
		{ 9-16	15.2	2.27		{ 38	37			
Do.....	Complete.....	{ 17-24	184.0	14.71	{ 15.25	{ 67	72	{ 1.51	{ 1.94	.226
		{ 25-32	191.5	15.78		{ 69	62			
Complete.....	.....do.....	{ 33-40	270.0	21.92	{ 20.59	{ 77	87	{ 1.87	{ 1.92	.184
		{ 41-48	255.0	19.26		{ 76	84			

<sup>1</sup> The ratio of roots to tops is taken as the weight of dry roots divided by the weight of dry stalks and leaves.

TABLE V.—Nitrogen and phosphoric acid absorbed by corn in Experiment II

Flask No.	Nitrogen (N) in dry stalks and leaves.	Nitrogen (N) in dry roots.		Nitrogen (N) absorbed by 8 plants.	Nitrogen (N) absorbed per gram of roots.	Phosphoric acid ( $P_2O_5$ ) in dry stalks and leaves.
		A-flasks.	B-flasks.			
	Per cent.	Per cent.	Per cent.	Gm.	Gm.	Per cent.
1-16	1.00	0.79	0.79	.....	.....	1.80
17-32	3.66	1.48	2.72	0.6022	0.310	1.28
33-48	3.57	2.46	2.46	.7973	.210	1.26

The partial-nitrogen plants made noticeably less growth than the normal plants, had 23 per cent greater ratio of roots to tops, absorbed 0.76 of the normal amount of nitrogen, and absorbed 1.48 times as much nitrogen per gram of roots as the normal plants. These figures agree remarkably well with those obtained in the previous experiment with rice.

#### EXPERIMENT III.—RICE WITH HALF THE ROOTS IN NITROGEN-FREE SOLUTION

##### EFFECT OF INCREASING CONCENTRATION OF NITROGEN

A test was conducted comparing the ordinary neutral solution with one containing twice the amount of nitrogen, to make sure the previous results were not influenced by a scarcity of nitrogen and to observe the effect on absorption of increasing the nitrogen.

The double-nitrogen solution was the same as the complete solution, except that the quantities of both potassium nitrate and sodium nitrate were doubled. Ferric citrate was used as the source of iron. The plants without nitrogen were grown in 200-c. c. flasks for 40 days; all others were grown in 200-c. c. flasks for 18 days, and in 500-c. c. flasks for the last 22 days. Eight flasks were taken as a unit and the units duplicated for each treatment. The solutions were changed eight times during the experiment. Experimental data are given in Tables VI and VII.

TABLE VI.—Growth of rice in Experiment III

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
			Gm.	Gm.	Gm.			Gm.	Gm.	
Nitrogen-free.....	Nitrogen-free.....	1-8	1.63	0.45	0.47	51	33	0.14	0.12	0.553
		9-16	1.77	.49		56	44			
Do.....	Complete.....	17-24	91.38	12.53	12.60	205	227	1.69	2.00	.293
		25-32	89.99	12.67		230	225			
Do.....	Complete double nitrogen.	33-40	93.25	12.40	12.69	209	213	1.60	1.86	.273
		41-48	93.30	12.08		210	204			
Complete.....	Complete.....	49-56	114.84	15.18	16.32	242	237	2.09	1.84	.241
		57-64	129.95	17.45		289	245			
Complete double nitrogen.	Complete double nitrogen.	65-72	145.35	19.54	18.00	280	274	2.25	2.13	.243
		73-80	121.47	16.45		266	267			

TABLE VII.—*Nitrogen and phosphoric acid absorbed by rice in Experiment III*

Flask No.	Nitrogen (N) in dry stalks and leaves.	Nitrogen (N) in dry roots.		Nitrogen (N) absorbed by 16 plants.	Nitrogen (N) absorbed per gram of roots.	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) in dry stalks and leaves.
		A-flasks.	B-flasks.			
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Per cent.</i>
1-16	1.03	0.93	0.93	.....	.....	.....
17-32	4.35	1.38	2.41	0.6124	0.306	1.50
33-48	4.49	1.47	2.60	.6344	.341	1.55
49-64	4.55	2.35	2.35	.8277	.211	1.63
65-80	4.52	2.35	2.35	.9093	.208	1.60

A comparison of plants 49 to 64 with 65 to 80 shows that, when all the roots of the plants were in the complete solution, doubling the nitrogen in the solution had no effect on the ratio of roots to tops, the nitrogen content of the plants, nor the amount of nitrogen absorbed per gram of roots. The slight increase in the weight of tops for 65 to 80 and the consequent increase in the total amount of nitrogen absorbed is of doubtful significance, as this increase is no greater than the variation between duplicates of the same lot.

A comparison of plants 17 to 32 with 33 to 48 shows that doubling the nitrogen in the solution, when the plants had only half their roots in the solution, had no effect on the growth of tops, but lowered the ratio of roots to tops, very slightly increased the total amount of nitrogen absorbed, and noticeably increased the amount of nitrogen absorbed per gram of roots. It is evident from these comparisons that, with the ordinary complete solution, the absolute amount of nitrogen supplied was sufficient for all the plants, and the previous results obtained were not due to a scarcity of nitrogen.

It follows from the preceding comparisons that doubling the nitrogen in the solution did not enable the partial-nitrogen plants to approach appreciably nearer the maximum nitrogen absorption (the amount of nitrogen absorbed by plants with all roots in the complete solution), although it did enable them to absorb more nitrogen per gram of roots. These facts are more evident from the following calculations: Plants 17 to 32 absorbed 0.74 as much nitrogen as 49 to 64, had 22 per cent greater ratio of roots to tops, and absorbed 1.45 times as much nitrogen per gram of roots, while plants 33 to 48 absorbed 0.77 as much nitrogen as 49 to 64, had 13 per cent greater ratio of roots to tops, and absorbed 1.62 times as much nitrogen per gram of roots.

#### EXPERIMENT IV.—RICE WITH THREE-FOURTHS THE ROOTS IN NITROGEN-FREE SOLUTION

It was important to see whether the rate at which the roots absorbed nitrogen could be still further increased by decreasing the number of roots in the complete nutrient solution. Accordingly in this experiment one lot of plants was grown with approximately three-fourths of its

roots in the nitrogen-free solution and one-fourth in the complete solution.

The nutrient solutions used were the same as those in Experiments I and II. The no-nitrogen plants were grown 30 days in 200-c. c. flasks; all others, 27 days in 200-c. c. flasks and 13 days in 500-c. c. flasks. Six flasks were taken as a unit and the units triplicated for each treatment. Solutions were changed eight times during the experiment. Experimental data are given in Tables VIII and IX.

TABLE VIII.—*Growth of rice in Experiment IV*

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
			Gm.	Gm.	Gm.			Gm.	Gm.	
Nitrogen-free.....	Nitrogen-free.....	1-6	1.50	0.36	0.37	54	55	0.148	0.147	0.797
		7-12	1.47	.37		44	43			
		13-18	1.49	.38		45	58			
Do.....	Complete.....	19-24	53.11	8.44	8.30	247	75	2.732	1.025	.453
		25-30	47.15	7.03		191	80			
		31-36	53.92	8.83		259	72			
Complete.....	do.....	37-42	85.70	12.27	12.41	212	187	2.103	2.132	.341
		43-48	84.83	12.41		198	207			
		49-54	85.66	12.55		188	212			

TABLE IX.—*Nitrogen and phosphoric acid absorbed by rice in Experiment IV*

Flask No.	Nitrogen (N) in dry stalks and leaves.	Nitrogen (N) in dry roots.		Nitrogen (N) absorbed by 12 plants.	Nitrogen (N) absorbed per gram of roots.	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) in dry stalks and leaves.
		A-flasks.	B-flasks.			
	Per cent.	Per cent.	Per cent.	Gm.	Gm.	Per cent.
1-18	1.33	0.50	0.50	.....	.....	.....
19-36	3.72	1.37	2.38	0.3642	0.355	1.60
37-54	4.63	2.47	2.47	.6666	.157	1.60

If we assume that plants 37 to 54 were normal in growth and composition, it appears that plants 19 to 36, with one-fourth of their roots in the complete solution, made about two-thirds the normal growth, had a 33 per cent higher ratio of roots to tops, absorbed 0.55 of the normal amount of nitrogen, and, per gram of roots, absorbed 2.26 times the normal amount of nitrogen.

The plants with one-half their roots in the complete solution in Experiments I, II, and III absorbed, respectively, 0.77, 0.76, and 0.74 as much nitrogen as the normal plants, and absorbed, respectively, 1.45, 1.48, and 1.45 times as much nitrogen per gram of roots as the normal plants. A comparison of the results of Experiments I, II, and III with those of this experiment shows that the smaller the portion of roots supplied with nitrogen, the less nearly the plants reach the maximum absorption of

nitrogen and the greater is the amount of nitrogen absorbed per gram of roots.

EXPERIMENT V.—RICE WITH ONE-HALF THE ROOTS IN A PHOSPHORUS-FREE SOLUTION

The previous experiments yielded pretty definite figures for the relative amounts and rates of nitrogen absorption for plants partially and completely supplied with nitrogen. It was therefore of interest to see whether the same figures would hold for the absorption of other mineral elements under like conditions. In this experiment the absorption of phosphorus was tested.

The complete neutral nutrient solution was used with ferric citrate as the source of iron. The double-phosphorus solution was the same as the complete, except the quantities of both dipotassium phosphate ( $K_2HPO_4$ ) and monopotassium phosphate ( $KH_2PO_4$ ) were doubled. The no-phosphate plants were grown in 200-c. c. flasks for 40 days; all others in 200-c. c. flasks for 26 days, and in 500-c. c. flasks for 14 days. Eight flasks were taken as a unit and the units duplicated for each treatment. The solutions were changed nine times during the experiment. Experimental data are given in Tables X and XI.

TABLE X.—Growth of rice in Experiment V

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
			Gm.	Gm.	Gm.			Gm.	Gm.	
Phosphorus-free . .	Phosphorus-free..	{ 1-8 9-16 17-24	{ 6.03 6.11 192.86	{ 1.96 1.94 30.25	{ 1.95 30.26	{ 120 129 139	{ 127 139 286	{ 0.56 4.80	{ 0.74 4.32	{ 0.666 .301
Do.....	Complete.....	{ 25-32 33-40	{ 195.01 208.37	{ 30.26 30.87	{ 29.53	{ 307 297	{ 292 256	{ 4.80 4.25	{ 4.32 4.14	{ .301 .284
Do.....	{ Complete double phosphorus.	{ 41-48 49-56	{ 186.44 202.36	{ 28.19 29.95	{ 29.34	{ 281 294	{ 258 258	{ 4.25 3.72	{ 4.14 3.54	{ .284 .247
Complete.....	Complete.....	{ 57-64 65-72	{ 192.20 181.78	{ 28.72 27.33	{ 28.09	{ 264 237	{ 267 236	{ 3.72 3.44	{ 3.54 3.54	{ .247 .248
Complete double phosphorus.	Complete double phosphorus.	{ 73-80	{ 189.34	{ 28.85		{ 250	{ 247			

TABLE XI.—Phosphoric acid and nitrogen absorbed by rice in Experiment V

Flask No.	Phosphoric acid ( $P_2O_5$ ).			Nitrogen (N) in dry stalks and leaves.
	In dry leaves and stalks.	Absorbed by 16 plants.	Absorbed per gram of roots.	
	Per cent.	Gm.	Gm.	Per cent.
1-16	0.17			
17-32	1.13	0.3386	0.0784	4.13
33-48	1.19	.3481	.0841	4.18
49-64	1.53	.4456	.0614	4.43
65-80	1.53	.4205	.0611	4.12



Phosphorus could not be determined accurately in roots which had grown in complete solutions, because of an adhering precipitate of ferric phosphate. Hence, in calculating the amount of phosphorus absorbed by the plants, account could be taken only of that present in the tops; the absolute figures for "grams of phosphorus pentoxid ( $P_2O_5$ ) absorbed per 16 plants" and "grams of phosphorus pentoxid ( $P_2O_5$ ) absorbed per gram of roots," in this and the following experiment, are thus considerably below the true values. The relative absorption for the different lots of plants is pretty well expressed by these figures, however, as the amount of phosphorus in the roots ought to be in fairly constant proportion to the amount in the tops, except that in plants 17 to 48 it is probably a little less than in 49 to 80, as plants 17 to 48 have some roots in the phosphorus-free solution.

If plants 49 to 64 are regarded as normal in growth and phosphorus content, it is evident that the partial-phosphorus plants, 17 to 32, made the same growth of tops as the normal plants, had 22 per cent greater ratio of roots to tops, absorbed 0.76 of the normal amount of phosphorus, and absorbed 1.28 times as much phosphorus per gram of roots as the normal plants.

Doubling the phosphates in the solution had about the same effect on the plants as doubling the nitrogen in Experiment III. When all the roots were in the complete solution, doubling the phosphates in the solution did not appreciably affect the growth of tops, the ratio of roots to tops, the amount of phosphorus absorbed, or the amount of phosphorus absorbed per gram of roots. In the case of plants with half their roots in the complete solution, doubling the phosphates in the solution had the effect of decreasing the ratio of roots to tops, very slightly increasing the amount of phosphorus absorbed, and increasing the amount of phosphorus absorbed per gram of roots.

#### EXPERIMENT VI.—RICE WITH TWO-THIRDS THE ROOTS IN PHOSPHORUS-FREE SOLUTION

To observe the effect of further decreasing the number of roots in the complete solution, a test was conducted in which one-third of the roots were maintained in the complete solution and two-thirds in the phosphorus-free solution.

The complete acid solution and the phosphorus-free solution were used, with ferric tartrate as the source of iron. The number of flasks per unit was the same as in the preceding test. The no-phosphate plants were grown in 200-c. c. flasks for 40 days; all others, in 200-c. c. flasks for 24 days and 500-c. c. flasks for 16 days. The solutions were changed nine times during the experiment. Experimental data are given in Tables XII and XIII.

TABLE XII.—Growth of rice in Experiment VI

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
Phosphorus-free...	Phosphorus-free..	{ 1-8 9-16 17-24 25-32 33-40 41-48	Gm. 5.53 5.94 279.07 269.01 281.28 284.12	Gm. 1.67 1.75 39.58 39.94 38.22 38.50	Gm. 1.71 39.76 38.36	{ 128 132 595 647 550 534	{ 144 135 203 275 301 285	Gm. 0.70 7.98 6.26	Gm. 0.78 3.86 3.28	.865 .298 .249
Do.....	Complete.....									
Complete.....	.....do.....									

TABLE XIII.—Phosphoric acid and nitrogen absorbed by rice in Experiment VI

Flask No.	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ).			Nitrogen (N) in dry stalks and leaves.
	In dry leaves and stalks.	Absorbed by 16 plants.	Absorbed per gram of roots.	
	Per cent.	Gm.	Gm.	Per cent.
1-16	.....	.....	.....	1.49
17-32	0.87	0.3459	0.0896	3.85
33-48	1.39	.5332	.0559	3.92

If we assume that plants 33 to 48 were normal, 17 to 32, with one-third their roots in the complete solution, made a normal growth, had a 20 per cent higher ratio of roots to tops, absorbed 0.65 of the normal amount of phosphorus, and absorbed 1.60 times the normal amount of phosphorus per gram of roots.

Decreasing the number of roots in the complete solution had about the same effect on phosphorus absorption that it did on nitrogen absorption—that is, it decreased the total amount of phosphorus absorbed and increased the phosphorus absorbed per gram of roots. The fractions of the normal amount of phosphorus absorbed by the plants with one-half and one-third their roots in the complete solution were, respectively, 0.76 and 0.65. These figures are in good agreement with similar factors of 0.76 and 0.55 for nitrogen absorption by plants with one-half and one-fourth of their roots in the complete solution. Per gram of roots, however, the partial-phosphorus plants increased their phosphorus absorption less than the partial-nitrogen plants increased their nitrogen absorption. This was due to the fact that growths made by the partial- and complete-phosphorus plants were about equal, while the partial-nitrogen plants made less growth than the complete-nitrogen plants.

The fact that plants with only part of their roots in the complete solution made about the same growth as the normal plants, although they contained considerably less phosphoric acid, shows that the normal plants absorbed considerably more phosphorus than they needed.

It is evident that under the special conditions of these tests the absorption of phosphorus and nitrogen obey practically the same law.

EXPERIMENT VII.—RICE WITH ONE-HALF THE ROOTS IN POTASSIUM-FREE SOLUTION

It was thought that the figures for the relative absorption of potassium by plants partially and completely supplied with potassium might vary somewhat from the similar figures for nitrogen and phosphorus, since, as is well known, sodium can to a small extent replace or supplement a deficiency of potassium in the plant, while a deficiency of nitrogen or phosphorus can not be supplemented by other mineral elements.

In this experiment the complete acid solution and the potassium-free solutions shown in Table I were used, while ferric tartrate was the source of iron. Eight flasks were taken as a unit and the units duplicated for each treatment. The plants were grown for 20 days in 200-c. c. flasks and 20 days in 500-c. c. flasks. Solutions were changed 10 times during the experiment. Experimental data are shown in Tables XIV and XV.

TABLE XIV.—Growth of rice in Experiment VII

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
Potassium-free....	Potassium-free....	1-8	Gm.	Gm.	Gm.	225	252	Gm.	Gm.	
		9-16	57.25	11.02	10.64	229	247	1.64	1.84	.327
		17-24	55.86	10.25		438	467			
Do.....	Complete.....	25-32	281.43	41.05	40.14	437	449	5.47	5.70	.278
		33-40	282.93	39.23		501	498			
Complete.....	.....do.....	41-48	316.60	43.12	42.77	514	469	5.92	5.51	.267
			299.40	42.42						

TABLE XV.—Potash and soda absorbed by rice in Experiment VII

Flask No.	Potash (K <sub>2</sub> O).					Soda (Na <sub>2</sub> O).				
	In dry stalks and leaves.	In dry roots.		Absorbed by 16 plants.	Absorbed per gram of roots.	In dry stalks and leaves.	In dry roots.		Present in 16 plants.	Absorbed per gram of roots.
		A-flasks.	B-flasks.				A-flasks.	B-flasks.		
Per cent.	Per cent.	Per cent.	Gm.	Gm.	Per cent.	Per ct.	Per ct.	Gm.	Gm.	
1-16	0.45	0.43	0.43	.....	.....	1.66	1.24	1.24	.....	.....
17-32	3.90	1.09	1.35	1.639	0.288	.78	1.48	1.39	0.4733	0.0424
33-48	5.25	2.76	2.76	2.498	.219	.71	.88	.88	.4043	.0354

Assuming that plants 33 to 48 were normal in growth and composition, it can be seen that the partial-potassium plants (17 to 32) had 4 per cent greater ratio of roots to tops, absorbed 0.66 of the normal amount of

potash, and absorbed 1.32 times the normal amount of potash per gram of roots. The plants partially supplied with potash absorbed considerably more soda than those completely supplied with potash. The partial-potassium plants absorbed 0.73 as much potash and soda combined as the complete-potassium plants.

EXPERIMENT VIII.—RICE WITH THREE-FOURTHS THE ROOTS IN POTASSIUM-FREE SOLUTION

As the figures for potash absorbed by the partial-potassium plants differed somewhat from similar figures for nitrogen and phosphorus, it was important to observe the potash absorption of plants with more roots in the potassium-free solution.

In regard to nutrient solutions, numbers of flasks per unit, and time the plants were grown in the different-sized flasks, this experiment was the same as the preceding. Solutions were changed 11 times during the experiment. Experimental data are given in Tables XVI and XVII.

TABLE XVI.—Growth of rice in Experiment VIII

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
Potassium-free....	Potassium-free....	{ 1-8 9-16 17-24 25-32	Gm. 46.50 47.66 216.98	Gm. 8.88 9.08 30.46	Gm. 8.98 30.87	{ 204 187 548 560	{ 213 201 254 222	Gm. 1.37 6.38	Gm. 1.47 2.62	.316 .292
Do.....	Complete.....	{ 33-40 41-48	Gm. 220.27 248.06 249.01	Gm. 31.27 33.80 34.85	Gm. 34.33	{ 572 579	{ 262 279	Gm. 6.87	Gm. 3.13	.291
Complete.....	.....do.....									

TABLE XVII.—Potash and soda absorbed by rice in Experiment VIII

Flask No.	Potash (K <sub>2</sub> O).					Soda (Na <sub>2</sub> O).				
	In dry stalks and leaves.	In dry roots.		Absorbed by 16 plants.	Absorbed per gram of roots.	In dry stalks and leaves.	In dry roots.		Present in 16 plants.	Absorbed per gram of roots.
		A-flasks.	B-flasks.				A-flasks.	B-flasks.		
	Per cent.	Per cent.	Per cent.	Gm.	Gm.	Per cent.	Per ct.	Per ct.	Gm.	Gm.
1-16	0.47	0.33	0.33	.....	.....	1.62	1.12	1.12	.....	.....
17-32	4.15	1.06	1.95	1.3482	0.515	.72	1.55	1.00	0.3473	0.0386
33-48	5.76	2.76	2.76	2.2018	.220	.35	.65	.65	.1851	.0185

Compared with the complete-potassium plants, the partial-potassium plants made slightly less growth, had the same ratio of roots to tops, absorbed 0.61 as much potassium, and absorbed 2.34 times as much potassium per gram of roots. The fact that partial-potassium plants

absorbed, with 29 per cent of their roots, 0.61 as much potassium as the complete plants is in good agreement with the results for nitrogen and phosphorus, where, with 27 per cent and 33 per cent of the roots, the absorptions were, respectively, 0.55 and 0.65.

In this and the preceding experiment the plants partially supplied with potassium absorbed considerably more sodium than plants completely supplied with potassium.

EXPERIMENT IX.—RICE WITH ONE-HALF THE ROOTS IN AN IRON-FREE SOLUTION (FERROUS SUPHATE SOURCE OF IRON)

As nitrogen, phosphorus, and potash are similar in being used by plants in relatively large amount, it was important to test the absorption of a mineral element used by the plant in small amount. Iron was used in this test, as this element is present in most plants in relatively minute quantities and as a deficiency markedly affects growth.

In this test approximately half the roots of the partial-iron plants were maintained in the iron-free solution. Both acid and neutral nutrient solutions were used. Ferrous sulphate was the source of iron, used so as to supply 0.008 gm. of iron per liter during the first 10 days of the test, 0.004 gm. of iron per liter during the next 10 days, and 0.002 gm. of iron per liter during the last 20 days. Four flasks were taken as a unit and the units triplicated for each treatment. The no-iron plants were grown in 200-c. c. flasks for 40 days; all others in 200-c. c. flasks for 27 days, and 500-c. c. flasks for 13 days. Solutions were changed six times during the experiment. Experimental data are given in Tables XVIII and XIX.

TABLE XVIII.—Growth of rice in Experiment IX

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	
			Gm.	Gm.	Gm.	Gm.	Gm.	
Iron-free neutral . .	Iron-free acid. . .	1-4	1.42	0.26	0.26	0.057	0.041	0.377
		5-8	1.39	.26				
		9-12	1.32	.26				
Complete neutral . . . . .	do . . . . .	13-16	20.62	4.10	4.38	.632	.547	.269
		17-20	33.80	4.78				
		21-24	31.19	4.27				
Do . . . . .	Complete neutral	25-28	49.25	6.34	6.61	.989	.772	.266
		29-32	53.67	7.00				
		33-36	50.49	6.48				
Iron-free neutral . .	Complete acid. . .	37-40	36.10	4.78	4.60	.539	.722	.274
		41-44	38.55	5.09				
		45-48	28.85	3.93				
Complete acid . . . . .	do . . . . .	49-52	48.42	5.99	6.27	.902	.984	.301
		53-56	48.70	6.22				
		57-60	51.97	6.61				



TABLE XIX.—Iron and nitrogen absorbed by rice in Experiment IX

Flask No.	Iron ( $\text{Fe}_2\text{O}_3$ ).			Nitrogen (N) in dry stalks and leaves.
	In dry stalks and leaves.	Absorbed by 8 plants.	Absorbed per gram of roots.	
	Per cent.	Gm.	Gm.	Per cent.
I-12	0.0400	.....	.....	3.90
13-24	.0229	0.000899	0.00142	3.90
25-36	.0223	.001370	.00078	3.80
37-48	.0378	.001635	.00227	3.72
49-60	.0378	.002266	.00120	3.70

This was one of the preliminary experiments conducted in a study of the assimilation of iron by rice from certain nutrient solutions (5). It was found that ferrous sulphate, used at this diminishing rate of 0.008, 0.004, and 0.002 gm. of iron per liter, apparently did not furnish sufficient available iron for an optimum growth of the plants. Because of this insufficiency of iron it was to be expected that plants with half their roots in the complete solution would absorb only half as much iron as those with all their roots in the complete solution, the former having only half as much iron at their disposal as the latter. This would doubtless have been the case if the available iron had been a fixed quantity in the solution. Other work showed, however, that most of the iron was not present in true solution, that the quantity of iron available was probably dependent on the rate at which iron went into solution, and that this rate was influenced by the rate at which it was removed by the plant. As plants with only half their roots in the complete solution would absorb iron more quickly than plants with all their roots, they might absorb more than half the quantity of iron.

If plants 13 to 24 are compared with 25 to 36, where the iron was added to the neutral nutrient solution, it can be seen that the partial-iron plants absorbed 0.66 as much iron as the complete-iron plants and absorbed 1.82 times as much per gram of roots. If we compare plants 37 to 48 with 49 to 60, where the iron was added to the acid nutrient solution, it can be seen that the partial-iron plants absorbed 0.72 as much iron as the complete-iron plants and absorbed 1.89 times as much iron per gram of roots.<sup>1</sup> The ratio of roots to tops varied little between the partial- and complete-iron plants in either the acid or the neutral solution. The figures for the relative amounts of iron absorbed by the partial- and complete-iron plants agree fairly well with similar figures for the other nutrients.<sup>2</sup>

<sup>1</sup> Small amounts of iron from ferrous sulphate are more available in the acid than in the neutral solution.

<sup>2</sup> There is another source of doubt in this experiment aside from the insufficiency of iron referred to above. Both ferrous and ferric iron were doubtless present in the solutions, and the plants probably absorbed both forms of iron. It is pointed out in another place (5) that there may be a difference in the efficiency of these two forms of iron in the plant. At all events, in several experiments ferrous sulphate was a less efficient form of iron than ferric compounds. The much higher percentage of iron in the plants grown in the acid solution is probably due to a greater absorption of ferrous iron.

EXPERIMENT X.—RICE WITH HALF THE ROOTS IN AN IRON-FREE SOLUTION (FERRIC TARTRATE SOURCE OF IRON)

The results of the previous experiment were not decisive, as there was a strong probability that neither the complete- nor the partial-iron plants had a sufficiency of iron available in the solution. Previous work showed, however, that in the acid solution with 0.008 gm. of iron per liter from ferric tartrate, plenty of iron was available; in fact, rice absorbed a certain excess of iron from this solution. Accordingly a test was run, using the acid solution with ferric tartrate.

In this test no plants were grown without iron, as the previous test and many others (5) showed that the growth of such plants was practically nil. Therefore, it would not appreciably affect the figure for the relative amounts of iron absorbed by partial- and complete-iron plants whether the quantity of iron in the no-iron plants was subtracted or not. The plants were grown 24 days in 200-c. c. flasks and 16 days in 500-c. c. flasks. Seven flasks were taken as a unit and the units duplicated for each treatment. Solutions were changed 10 times during the experiment. The results are given in Tables XX and XXI.

TABLE XX.—Growth of rice in Experiment X

Nutrient solution.		Flask No.	Weight of stalks and leaves.			Number of roots.		Average oven-dry weight of roots.		Ratio of roots to tops.
A-flasks.	B-flasks.		Green.	Oven-dry.	Average oven-dry.	A-flasks.	B-flasks.	A-flasks.	B-flasks.	
Iron-free .....	Complete. ....	{ 1-7 8-14 15-21 22-28	Gm. 260.5 277.1 304.3 320.8	Gm. 37.11 40.46 43.23 46.79	Gm. 38.79 45.01	{ 392 383 358 396	{ 379 361 378 403	Gm. 4.23 5.53	Gm. 5.38 5.95	0.248 255
Complete. ....	do. ....									

TABLE XXI.—Iron, nitrogen, and phosphoric acid absorbed by rice in Experiment X

Flask No.	Iron (Fe <sub>2</sub> O <sub>3</sub> ).			Nitrogen (N) in dry stalks and leaves.	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) in dry stalks and leaves.
	In dry stalks and leaves.	Absorbed by 14 plants.	Absorbed per gram of roots.		
1-14	Per cent. 0.0189	Gm. 0.00733	Gm. 0.00136	Per cent. 3.64	Per cent. 1.34
15-28	0.0246	0.01107	0.00096	3.53	1.33

Comparison with the complete-iron plants (15 to 28) shows that the partial-iron plants (1 to 14) absorbed 0.66 as much iron and 1.43 times as much iron per gram of roots. The ratio of roots to tops and the percentages of nitrogen and phosphoric acid in the dry substance varied little between the two lots of plants

## SUMMARY OF EXPERIMENTAL RESULTS

The essential results of the 10 preceding experiments are summarized in Table XXII.

TABLE XXII.—*Summary of results of Experiments I to X*

Experiment No.	Plant grown.	Element tested.	Complete nutrient solution.	Proportion of roots in complete solution.	Total amount of element absorbed. <sup>a</sup>	Amount of element absorbed per gram of roots. <sup>a</sup>	Ratio of root to top. <sup>a</sup>
				<i>Per ct.</i>			
I.....	Rice.....	Nitrogen.....	Neutral.....	50	77	145	123
II.....	Corn.....	do.....	do.....	56	76	148	123
III.....	Rice.....	do.....	do.....	54	74	145	122
III.....	do.....	do.....	Neutral, double nitrogen.	54	77	162	113
IV.....	do.....	do.....	Neutral.....	27	55	226	133
V.....	do.....	Phosphorus.....	do.....	47	76	128	122
V.....	do.....	do.....	Neutral, double phosphorus.	50	78	137	115
VI.....	do.....	do.....	Neutral.....	33	65	160	120
VII.....	do.....	Potassium.....	Acid.....	51	66	132	104
VIII.....	do.....	do.....	do.....	29	61	234	100
IX.....	do.....	Iron.....	Neutral, ferrous sulphate.	54	66	182	102
IX.....	do.....	do.....	Acid, ferrous sulphate.	57	72	189	91
X.....	do.....	do.....	Acid, ferric tartrate..	56	66	143	97

<sup>a</sup> Total amount of element absorbed, amount absorbed per gram of roots, and root to top ratio, each taken as 100 for plants with all roots in complete solution. Corresponding values for plants with part of roots in complete solution expressed relative to 100.

The amount of nitrogen or phosphorus absorbed by plants with half their roots in the complete solution was 0.76 of that absorbed by plants with all their roots in the complete solution, while the similar figure for potassium and iron was 0.66. As already pointed out, it was to be expected that a somewhat different figure would be obtained for potassium than for nitrogen and phosphorus, as a lack of potassium can be supplemented to some extent by an abundance of sodium. The lower figure for iron may be connected with the immobility of iron in the plant (4).

The less absorption of an element supplied to only part of the roots of a plant was not due to the partial plants having a smaller absolute amount of the element available than the complete plants. This was proved by the results of Experiments III and V, where doubling the quantity of nitrogen or phosphorus in the solution supplied to half the roots did not appreciably increase the amounts of nitrogen or phosphorus absorbed by these plants.

It is apparent that the fewer roots in the complete solution the less nearly the plants reached the optimum absorption. The decrease from the optimum absorption was not, however, directly proportional to the portion of roots in the complete solution. The relation between the portion of roots in the complete solution and the fraction of the maximum amount of nitrogen absorbed is shown by the curve in figure 1.

The maximum amount of nitrogen, 100, is taken as that absorbed by plants with 100 per cent of their roots in the complete solution. As plants with no roots in a complete solution (the incomplete solution being nitrogen-free) can absorb no nitrogen, the zero point is also fixed. The two other points found experimentally for plants with 27 per cent and 53 per cent of their roots<sup>1</sup> in the complete solution determine fairly well the form of the curve.

From the uniformity of the preceding results it seems permissible to state, as a rule, that when all the roots of a plant are supplied with all the essential elements except one, the fewer the roots supplied with the one lacking element the less nearly the plant will attain a maximum absorption of this element. As this rule has been found valid for nitrogen, phosphorus, potassium, and iron, it probably applies to all the mineral nutrients. The rule holds when the total quantity of the element supplied is equal to or in excess of the needs of the plant. If the total quantity of element supplied is insufficient for the needs of the plant, a more equal absorption of the element by plants with part and with all their roots might be expected. This, however, has not been proved.

The relative amounts of the element absorbed per gram of roots by plants with part and with all their roots supplied with the element varied considerably, according to several conditions, although this figure was always much higher for the plants partially supplied than for those completely supplied. The greater the depression of growth by the smaller total amount of the element absorbed by the plants partially supplied, the greater was the quantity of the element absorbed per gram of roots. When the partially supplied plants had a greater ratio of roots to tops than the completely supplied plants, the absorption per gram of roots for the partial plants did not so greatly exceed that of the complete plants. The smaller the portion of roots in the complete solution, the greater was the absorption of the element per gram of roots in the complete solution.

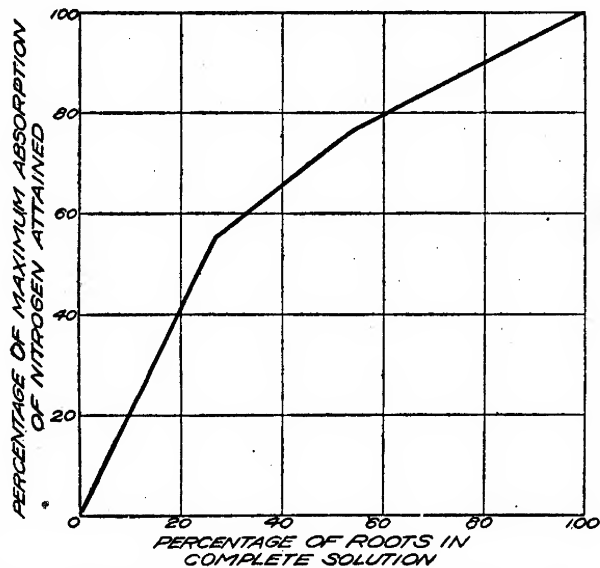


FIG. 1.—Relation between portion of roots supplied with nitrogen and portion of maximum absorption attained.

<sup>1</sup> The average of results from Experiments I to IV are used.

It was expected that plants whose growth was restricted by having a portion of the roots present in the incomplete solution would show an increased absorption of elements present in both the incomplete and complete solutions. The results did not justify the expectation, except in the case of plants partially supplied with potassium. In Experiments I, II, III, and IV the partial-nitrogen plants contained practically the same percentages of phosphoric acid as plants completely supplied with nitrogen. In Experiments V and VI the partial-phosphorous plants contained about the same percentages of nitrogen as the complete-phosphorous plants. In Experiments VII and VIII the partial-potassium plants contained considerably more sodium than the complete-potassium plants. And in Experiments IX and X the partial-iron plants contained practically the same percentages of nitrogen and phosphoric acid as the complete-iron plants.

It will be noted that, in all the experiments with nitrogen or phosphorus, plants with part of their roots in the complete solution had a considerably greater ratio of roots to tops than plants with all their roots in the complete solution. It might be thought that this was due to the incomplete solution furnishing a better medium for growth, aside from nutritive effects, than the complete solution. A calculation of the average weights of individual roots in the two solutions shows this assumption to be incorrect and suggests that the increased ratio of roots to tops of the partial plants was due to an adaptation of the plant under the stimulus of a deficiency of the element. The calculations show that, with plants partially supplied with nitrogen or phosphorus, the average weight of roots in the nitrogen- or phosphorus-free solution was slightly less in nearly every case than the weight of roots in the complete solution. Also the average weight of roots in the complete solution of plants with part of their roots in the complete solution was slightly greater than the weight of roots of plants with all their roots in the complete solution.

In experiments where potash or iron was the lacking element the plants with part of their roots in the complete solution did not have an appreciably different ratio of roots to tops from plants with all their roots in the complete solution. This was probably due to the fact that rice does not so readily respond, by greater root growth, to a certain deficiency of these elements. As the plants receiving absolutely no potassium or iron had a markedly increased ratio of roots to tops, it is apparent that the difference in the response to a lack of nitrogen and phosphorus or potassium and iron is chiefly one of degree.

#### DISCUSSION OF RESULTS

The two chief facts brought out in the preceding cultural tests are (1) that the fewer roots supplied with an ion the greater is the absorption of the ion per gram of roots, and (2) that a plant is unable to attain a



maximum absorption by means of only a portion of its roots. It is not felt that a fully adequate explanation can be given of these facts until more is known of the mechanism and dynamics of absorption, translocation, and utilization of mineral elements in the plant. The following explanation is offered, not as an hypothesis, but more as a suggestion of the general way in which the results may have been brought about.

Roughly it may be said that the absorption of a mineral element is dependent on utilization, that, as the ions are removed by formation of new compounds, etc., in the plant, more ions can be absorbed. Under the special conditions of the tests described here, absorption is primarily dependent on utilization, but the rate at which ions can be translocated from the absorbing cells to the utilizing cells also affects both utilization and absorption.<sup>1</sup> When one-half the roots of a plant are supplied with an ion, it may be said that the plant absorbs only three-fourths as much of the ion as when all the roots are supplied, because the rate of transference from the absorbing cells to the utilizing cells is diminished by one-fourth. The transference of ions from the cells where they are absorbed to the cells where they are utilized, of course, involves a series of translocations. In speaking, as is done above, of the rate of transference from absorbing to utilizing cell, the average rate of the whole series of translocations is understood.

While the total amount of an ion absorbed by a plant would thus be partially dependent on the rate at which the whole series of transferences from absorbing to utilizing cells proceeded, the quantity of an ion absorbed per gram of roots would depend, not on the rate of the whole series of translocations, but on the rate of translocation from the root cells. It can be supposed that the fewer the roots supplied with an ion, the faster will the translocation of the ion from the absorbing and root cells proceed, but the slower will be the rate of the whole series of translocations. Thus, when a portion of the roots are supplied with an ion, the amount absorbed per gram of roots may be greater and the total absorbed may be less than when all the roots of a plant are supplied with an ion. This is illustrated in figure 2.

In this figure *DE* represents the rate of transference in the plant when one-fourth the roots are supplied with an ion, and *AB* the rate when all the roots are supplied. The rate of transference at *O*, the absorbing cells, are respectively *DO* and *AO*, *DO* being 2.26 times *AO*. The average rates of the whole curves are  $\frac{ODEC}{OC}$  and  $\frac{OABC}{OC}$ , the former being 0.55 of the latter.

As the conditions of the preceding experiments were ideal in the sense that there were sufficient available mineral nutrients at all times, the results were due to the way the plant functions in absorbing mineral elements. The results are, therefore, to a certain extent applicable to soil conditions and have a direct practical bearing.

<sup>1</sup> Utilization and absorption are, of course, reciprocally dependent.

It is obviously important to apply fertilizers in such a way that, so far as possible, all the roots of the plants will be supplied with the fertilizing element. The lateral diffusion of fertilizer salts in the soil being small, this can best be done by distributing the fertilizer uniformly over the whole area occupied by the roots. Whether a field plant will, with a portion of its roots, absorb all the fertilizing element applied depends on whether the quantity applied is sufficient for the needs of the plant and on various actions taking place in the soil, as leaching, fixation, etc. This work shows, however, that the optimum condition will be obtained by applying the fertilizer to all the roots. While the plant shows a degree of adaptation in its absorption as shown in these tests, it is not safe to assume that it will capture all the fertilizer wherever applied.

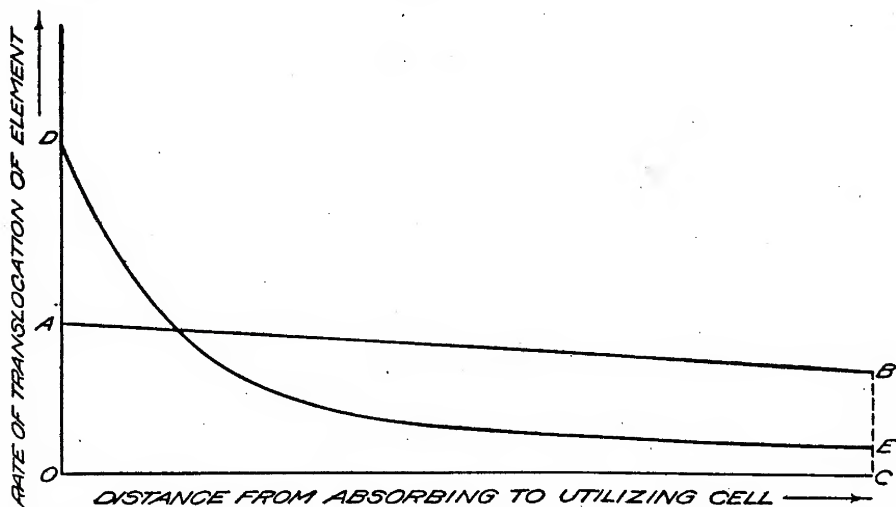


FIG. 2.—Possible relative rates of translocation of an element in plants with one-fourth and all their roots supplied with the element.

In the preceding work it was not shown whether a plant can absorb a maximum amount of an element when it is available to a certain fraction of all the roots, as the lower, upper, middle, or different parts of all the roots. It is to be expected, however, that the results obtained in this work would apply equally to this similar condition. If the idea is carried a step farther, it would also be expected that a maximum absorption would be attained only when all the absorbing cells of a root were supplied with the nutrient.

If the latter assumption is correct, a maximum absorption of the mineral nutrients from the soil would depend on all the nutrients being available throughout the soil. Since the plant for the most part obtains its nutrients from the aqueous film surrounding the soil particles, a theoretically maximum absorption would be obtained only when all the essential elements were present in all the soil films with which the roots came in contact. While this is plainly carrying the results actually

obtained pretty far afield, some of these assumptions appear to be substantiated by other work, the results of which it is hoped to present later.

The results of the preceding work have some bearing on the law of minimum. The curve in figure 1, showing the percentage of the maximum absorption of nitrogen attained when different percentages of the roots are supplied with nitrogen, has a form similar to that which Mitscherlich claims for the minimum curve (7, 8). The formula deduced by Mitscherlich for the way in which plant growth increases with increase of the minimum factor is—

$$\log. (A-y) = \log. (A-a) - K. x.$$

$x$  is the vegetative factor present in minimum. In our case it is the percentage of roots in the complete solution.

$y$  is the yield obtained for any value of  $x$ . In our case it is the total amount of the element absorbed by the plants with any portion of the roots in the complete solution. The amount of the element absorbed is expressed relative to 100, as in Table XXII.

$A$  is the maximum yield obtainable, under the conditions, by increase of  $x$ . In our case  $A$  will be the absorption attained when 100 per cent of the roots are in the complete solution. This is taken as 100 in each experiment.

$a$  is the yield obtaining without any addition of  $x$ . In our case  $a$  is obviously always 0, as there can be no absorption of the element when no roots are supplied.

$K$  is a constant, "the differential factor."

By the substitution of the values obtained in Experiment I, as shown in Table XXII, the equation would be—

$$\log. (100-77) = \log. (100-0) - K. 50$$

$$K. = 0.0128$$

By the use of the data of experiments on the absorption of nitrogen and phosphorus afforded by Experiments I to VI in Table XXII, the following values for  $K$  were obtained:<sup>1</sup>

0.0128  
.0111  
.0108  
.0118  
.0128  
.0128  
.0132  
.0138

Average value, 0.0124-±0.0003

<sup>1</sup> The values for the absorption of potash were not used on account of the probable partial replacement of potash by soda. Using the data for iron absorption in Experiments IX and X, the values for  $K$  are, respectively, 0.0087, 0.0097, and 0.0084, average 0.0089. The fact that a different value for  $K$  was obtained in the experiments on iron absorption does not mean that the formula fails to hold here, but merely expresses the difference, already noted, between the absorption of iron and nitrogen or phosphorus.

The value for  $K$  is sufficiently constant to create a strong probability that Mitscherlich's mathematical expression (7) represents the relation between the quantity of roots supplied with an element and the amount absorbed. The results may therefore be taken as further proof that Mitscherlich's formulation of the law of minimum is correct for ideal conditions.

#### SUMMARY

Tests were conducted in water cultures to see whether a plant could absorb a maximum amount of one mineral element which was supplied to only part of the roots if all other essential elements were supplied to all the roots. The absorption of nitrogen with rice and corn and of phosphorus, potassium, and iron with rice was tested in this way, one-half the roots being maintained in a nutrient solution lacking one of these elements. Tests were also conducted, varying the portion of roots in the complete and incomplete solutions.

The results show that, under the conditions described, the plant does not absorb a maximum amount of the element, and the fewer the roots supplied with the element, the smaller the total amount absorbed. This applies when the total amount of the element supplied is equal to or in excess of the needs of the plant. A curve was plotted showing approximately what portion of the maximum absorption can be expected with any fraction of the roots supplied with the element. With nitrogen and phosphorus the total amount absorbed by plants with half their roots in the complete solution was 0.76 of that absorbed by plants with all their roots in the complete solution. The similar figure for potassium or iron was 0.66. Increasing the concentration of the element in question in the complete solution did not appreciably alter the results.

The amount of the element absorbed per gram of roots increased greatly as the number of roots in the complete solution was diminished.

The results are explained on the basis of the rate of utilization and transference of the elements in the plant.

Attention is called to the bearing of these results on the method of applying fertilizers.

The results obtained agree with Mitscherlich's formulation of the law of minimum.

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## FLOW THROUGH SUBMERGED RECTANGULAR ORIFICES WITH MODIFIED CONTRACTIONS<sup>1</sup>

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### INTRODUCTION

The measurement of water flowing in open channels is a matter of growing importance, especially throughout the irrigated West, where the adoption of more economical methods of water delivery and canal management often is retarded by a lack of information concerning measuring devices adapted to specific field conditions. Where the use of free-flow weirs is prohibited by the low grade of canals and ditches, some type of submerged orifice has been substituted in many cases, though probably the majority of such ditches still are without any provision for measurement, except an occasional use of the current meter. The majority of the orifices installed have had complete end and bottom contractions, the choice being due, no doubt, to the more extensive and reliable information available concerning orifices of this type. The principal objections to a submerged orifice with complete contractions are the cost of the structure and the fact that it is not adapted to the measurement of water that carries sand and silt. These factors have prevented the installation of many measuring devices, and many have been installed where accumulations of sand and silt have rendered the measurements either questionable or worthless.

The complete suppression of the bottom contraction and the partial suppression of the end contractions will give a velocity of approach that will prevent sand and silt troubles in the orifice box, and will also lessen the cost of the structure. This is practically what has been done on many irrigation systems where lateral head gates and farmers' turnouts have been used directly as a means of measuring the flow. There are innumerable sizes, shapes, and conditions of setting such structures,

<sup>1</sup> The work upon which this paper is based was done in the hydraulic laboratory, Fort Collins, Colo., under cooperative agreements between the Office of Experiment Stations and the Office of Public Roads and Rural Engineering, United States Department of Agriculture, and the Colorado Experiment Station.

there often being several kinds on a single canal system; but, as few of them have been calibrated, their value as measuring devices is not great. Of the few experiments made upon submerged orifices with modified contractions, only a small percentage are comparable to irrigation conditions.

A series of experiments was made in the hydraulic laboratory at Fort Collins, Colo., during the summer of 1914, for the purpose of developing some form of submerged orifice that would meet practical conditions.<sup>1</sup> It was practically impossible to make experiments upon all the different arrangements of structures used in the field, because of their infinite number and the further fact that many of them are essentially not adapted

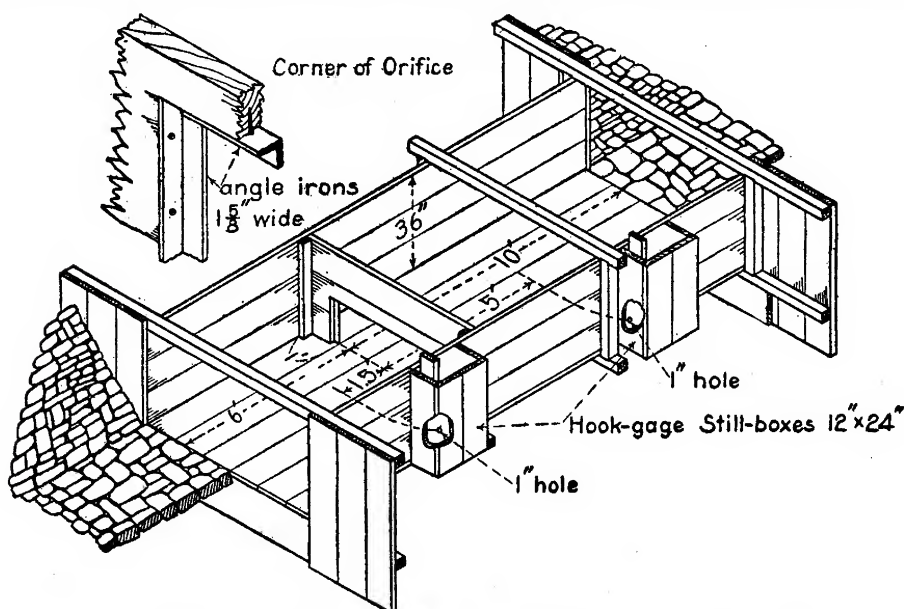


FIG. 1.—Standard box for submerged rectangular orifices with modified contractions.

to the measurement of water with a reasonable accuracy. Obviously it was necessary to work toward a standardization of the dimensions and arrangement of the orifice and orifice box in order that it might meet as nearly as possible the many conditions under which it would have to be installed in the field and still give a discharge to conform to a general formula or table. Several series of experiments were made before a set of conditions was decided upon as the standard.

The standard is a simple arrangement of orifice without bottom contraction but with angle-iron sides and top, and with end contractions of 1 foot (fig. 1). In this form it is a measuring device exclusively, but it may be combined with a gate, as indicated in Table I and the

<sup>1</sup> For a description of the hydraulic laboratory, see the following:

Cone, V. M. Hydraulic laboratory for irrigation investigations, Fort Collins, Colo. *In Engin. News*, v. 70, no. 14, p. 662-665, 5 fig. 1913.

— Flow through weir notches with thin edges and full contraction. *In Jour. Agr. Research*, v. 5, no. 23, p. 1051-1113, 21 fig. 1916.

accompanying text figures, and a corresponding correction applied to the discharge table. The corrections are given for several different arrangements of metal and of wood gate guides, metal and wood edges of the orifice, and with and without a bottom contraction strip such as often is used for a bottom gate stop. It is expected that with these data many engineers and canal managers can arrange structures to meet their local demands and still give a reasonably dependable measurement of the quantity of water passing through the orifices. If the orifice box is built of concrete, the cross wall in which the orifice is placed must be given a flaring enlargement downstream from the orifice, to allow lateral expansion of the issuing stream of water.

Although the submerged orifice is a means to a satisfactory solution of some practical problems, it can not well be classed with precise measuring devices. Its discharge is influenced by comparatively trivial factors the identity of which often is unknown. Sand and silt troubles are eliminated by modifying the end and bottom contractions, but floating trash will accumulate in the orifice box. The new type of weir,<sup>1</sup> with suppressed bottom contraction, is entirely self-cleaning of trash, sand, and silt, and has practically the same accuracy as the modified orifice. It is therefore better to install the new weir where there is sufficient fall in the ditch to give free flow and where a separate headgate is provided.

Since there is a close relation between the accuracy of the measurement of flow and the accuracy of the determination of the head acting on the orifice, it is essential that some form of close-reading gage be used with the submerged orifice. The stilling well is necessary because of the comparatively high velocity of water in the orifice box:

#### ARRANGEMENT OF EXPERIMENTAL APPARATUS

The concrete weir box in the hydraulic laboratory is 6 feet deep, 10 feet wide, and 20 feet long, with a channel of approach about 60 feet long. By-passes in the side of the weir box permit a nice control of the water level. In an opening near the top and middle of the end wall of the weir box is placed a T-iron frame, 3 feet high by 6 feet long, in which the weir and orifice plates are placed for experimental purposes. In the series of experiments with orifices having modified contractions it was necessary to cover this opening with planks  $1\frac{5}{8}$  inches thick, made rigid and watertight. A floor of matched lumber was built in the concrete weir box about 3 feet above the bottom, as shown in figure 2. This floor was made level, rigid, and tight against the orifice bulkhead. Another floor, having a length of 6 feet, was placed on the downstream side of the orifice bulkhead, and made level with the floor on the upstream side.

The orifice was made by cutting a rectangular opening in the bulkhead so the bottom of the opening was at the floor line. The smallest orifice

<sup>1</sup> Cone, V. M. A new irrigation weir. *In Jour. Agr. Research*, v. 5, no. 24, p. 1127-1143, 16 fig. 1916.

was used first. Pieces of angle iron having a width of  $1\frac{5}{8}$  inches and a thickness of  $\frac{3}{16}$  inch, with an edge planed square, or strips of wood  $1\frac{5}{8}$  inches square, were inserted in the opening to be flush with the upstream face of the bulkhead. These strips were screwed to the bulkhead to make the orifice opening true, of the desired size, and to prevent leakage. The condition of orifice taken as the standard had angle-iron sides and

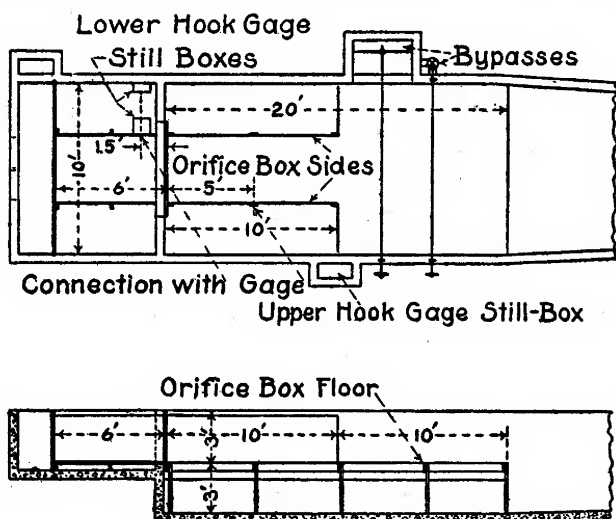


FIG. 2.—Plan, elevation, and section of orifice box in concrete channel.

top, but no bottom contraction (fig. 3), though other experiments were made with a similar arrangement of wood strips and with a bottom contraction of  $1\frac{5}{8}$  inches formed by the width of the angle iron or wood strip. There were also several arrangements of metal and wood gate guides (see fig. 3 to 11, inclusive).

A bulkhead placed across the channel, 6

feet downstream from the orifice bulkhead, contained a 20-inch square steel head gate the bottom of which was at the floor line. This gate was operated by a screw lift, which permitted a fair regulation of the water level downstream from the orifice; but the finer regulation was obtained by a 2-inch valve placed in the side of the channel.

Sections, 3 feet high and 10 feet long, made of matched lumber were used for the sides of the orifice box or channel of approach to the orifice. They could be moved to any position desired and fastened firmly to the floor. At the upstream end of the side sections, wings were attached at an angle of  $90^\circ$ , while the other end of the side sections butted against the orifice bulkhead. Although this box was practically water-tight, the question of leakage was of little importance because the box was entirely surrounded by water. Similar adjustable sides were placed downstream from the orifice and unless otherwise stated the widths of the channels of approach and recession were equal. In all the experiments the sides were set parallel and vertical and at an equal distance from the center line of the orifice.

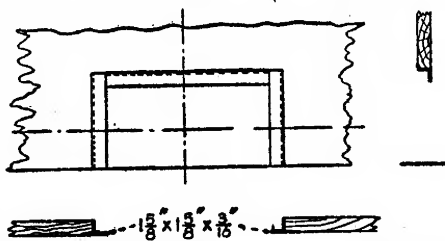


FIG. 3.—Elevation and sections of standard orifice without bottom contraction.



The head on the upstream side of the orifice was determined in the concrete still box by means of a standard type Boyden hook gage. The head was communicated to the still box through four lengths of  $\frac{3}{4}$ -inch hose connected to 1-inch pipe nipples placed through the side of the channel of approach until just flush with the inner face. These pipes were placed close together, so as to give an average distance of 5 feet from the plane of the orifice. The head on the upstream side of the orifice was kept constant by means of the by-passes and the main regulating gates at the storage reservoir.

The head on the downstream side of the orifice was measured with a hook gage placed in a metal still box anchored to the concrete wall. The velocity of recession was so great in some cases as to cause a pulsation in the still box, which prevented a reasonably accurate measurement of the height of the water level. This was satisfactorily overcome by placing a metal tank, 12 by 12 by 30 inches, between the hook gage still box and the channel of recession. This regulating tank was connected with the channel of recession by a 1-inch pipe nipple placed through the side of the channel 1.5 feet from the plane of the orifice and 0.5 foot above the floor. The regulating tank was connected to the still box by a single piece of  $\frac{3}{4}$ -inch hose about 2 feet long.

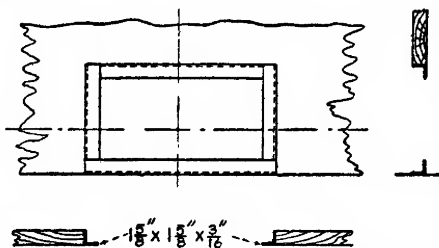


FIG. 4.—Elevation and sections of orifice with bottom contraction.

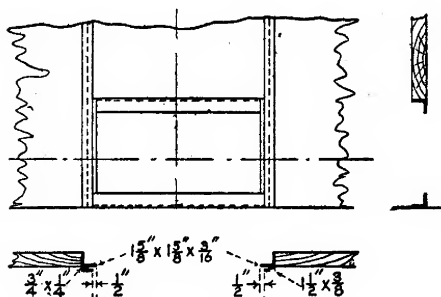


FIG. 5.—Elevation and sections of orifice with iron gate guides.

For each setting of orifice and arrangement of orifice box a number of observations, sufficient to determine the discharge curve, were made with different elevations of the water levels upstream and downstream from the orifice. The depth of water in the channel of approach remained constant for each set of observations, while the depth downstream was changed. However, no observation was started until the

desired conditions of flow had been secured, and these conditions were not allowed to vary during the observation. The volume of water which flowed through the orifice during each test was determined accurately in the calibrated concrete tanks.

The exact dimensions of the orifices were measured with a micrometer caliper before and after the experiment, and, where slight changes were caused by swelling of the wood, average dimensions were taken. Usually

there was no appreciable change, but occasionally there was a change amounting to a few ten-thousandths of an inch.

In some of the experiments, where the greatest quantities of water passed through the orifice, there was a tendency toward vortexes. The sur-

face of the water immediately upstream from the orifice would take a whirling motion with a greater or less depression, but the funnel never was sufficiently complete to allow air to be drawn through the orifice. The effect of these vortexes upon the discharge through the orifices was not apparent under the conditions of the experiments, but it

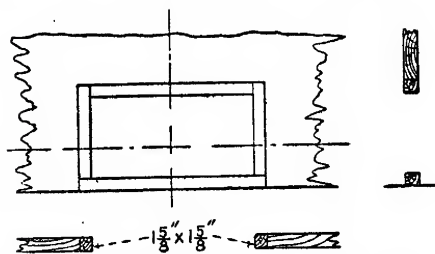


FIG. 6.—Elevation and sections of broad-edged orifice.

might amount to considerable with a smaller depth of water in the channel of approach.

#### STANDARD CONDITIONS AND DIMENSIONS FOR SUBMERGED RECTANGULAR ORIFICES WITH MODIFIED CONTRACTIONS

As a result of experiments with several different arrangements the conditions described below and illustrated in figures 12 and 13 were taken as the standard because they appear to give the most reliable results, meet the practical demands for a measuring device of this type, and reduce the cost of construction. It is essential that the orifices and orifice boxes be built according to these specifications if the discharge formula or table is to be used. The influence of various changes in the size of the box and arrangement of the orifice is shown in Table I.

The total length of the orifice box is 16 feet, 10 feet of which forms the channel of approach.

Wings set at an angle of  $90^\circ$  are attached to the sides of the upstream end of the orifice box. The floor of the box is level throughout and at the same elevation as the bottom of the canal. The box should be set in the center line of the canal, so as to allow the water to enter the box in straight lines. The sides are parallel and are placed apart a distance equal to the length of the orifice plus 2 feet. Orifices of all sizes have end-contraction distances of 1 foot.

The orifice must have sharp sides and top, and no bottom contraction. Angle irons  $1\frac{5}{8}$  inches wide were used in the experiments and were placed as shown in figure 3. The orifice must be placed with its greatest dimen-

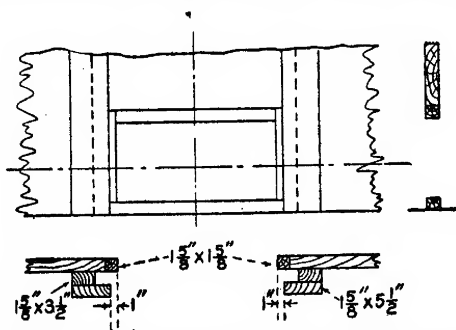


FIG. 7.—Elevation and sections of broad-edged orifice with wood gate guides.

sion horizontal. If it is desirable to use an orifice with bottom contraction, or with wood sides and top, or with gate guides and gate, the discharge tables may be corrected in accordance with the data given in Table I and the deductions from that table given on pages 106 to 108.

The elevations of the water levels in the channels of approach and recession should be taken in separate stilling boxes, one connection being 5 feet upstream and the other 1.5 feet downstream from the plane of the orifice. The connections should be through the side of the orifice box about 0.5 foot above the floor line.

The discharge tables were computed for a depth of 2.5 feet of water in the channel of approach. This depth was used in nearly all the standard experiments, the exceptions being with some of the smaller orifices, where a depth of 2.75 feet was used. This slight difference was not sufficient to change the discharge appreciably, because the velocity of approach was small in both cases.

Table I contains a summary of the results of the 317 observations with 60 different combinations of sizes of orifices, sharp

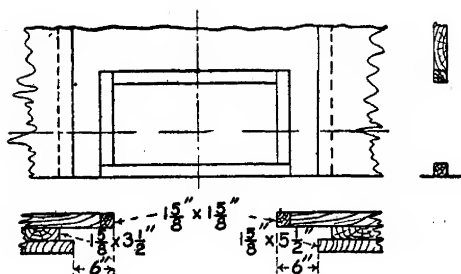


FIG. 9.—Elevation and sections of broad-edged orifice with wood gate guides moved out 6 inches.

and thick edges, with and without gate guides, with and without small bottom contraction, with different depths of water in the channel of approach, and with different end contractions in the channel of approach and recession. The data in the table and the figures referred to in the column to the right of the equations indicate the conditions under which each set of observations was made, with the exception of No. 49 and 50. No. 49 was with the sides of the channel of approach set at a width of 10 feet and the sides of the channel of recession set at a width of 3.0 feet, which gave end contractions of 0.5 foot. No. 50 was with the sides of both the channels of approach and recession set at a width of 10 feet. In all other cases the sides of the channel of approach and recession were set at an equal width.

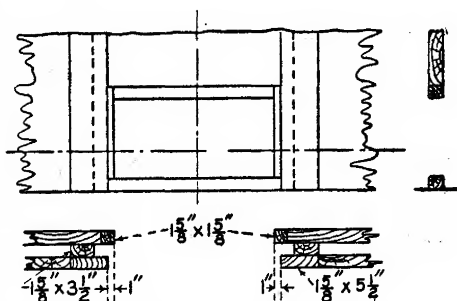


FIG. 8.—Elevation and sections of broad-edged orifice with wood gate guides and wood backing.

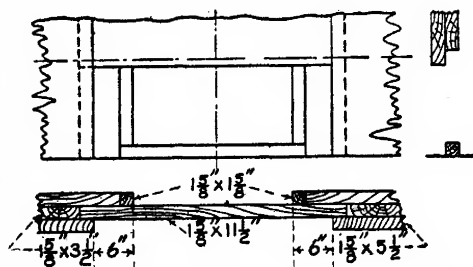


FIG. 10.—Elevation and sections of broad-edged orifice with wood gate slide and guides.

The words "with" or "without" in the column headed "Bottom contraction" indicate whether the floor formed the bottom of the orifice, or an angle iron or wood strip extended above the floor a distance of  $1\frac{5}{8}$  inches, such as would be used for a bottom gate stop.

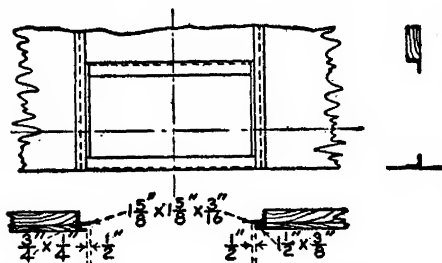


FIG. 11.—Elevation and sections of standard orifice with iron gate guides and backing.

the general formula for the standard conditions of orifices. The discharges computed from the general formula were taken as the basis of comparison, plus and minus signs representing greater and less discharges, respectively, by the individual formula than by the general formula. This arrangement allows the effects of various alterations in the size and setting of the orifice and orifice box to be compared more easily than would be possible from a number of complete discharge tables, and indicates the correction which should be applied to make the discharge tables applicable for each condition given.

The equations given in Table I were obtained from large-scale logarithmic plots of the experimental data for each arrangement of orifice and orifice box by the use of the discharges and differences in heads as the coordinates. The straight-line

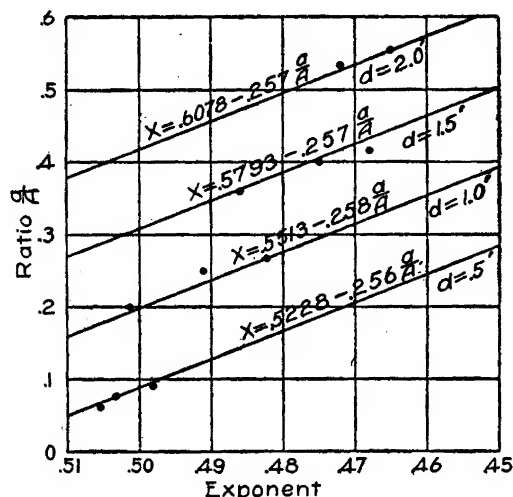


FIG. 12.—Plots of exponent values of equations in Table II

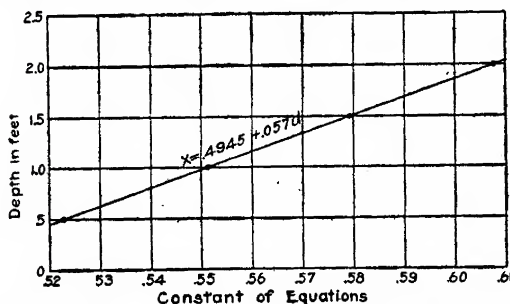


FIG. 13.—Plots of constants of equations given in figure 12.

that both exceptions were for high heads and for the same size of orifice, 0.5 by 2.0 feet, with and without angle-iron contraction in the bottom



of the orifice, and a duplication of the experimental work checked the results as given. No explanation is apparent for this isolated inconsistency.

TABLE I.—Summary of results of experiments, showing the influence of various changes in the size of the box and the arrangement of the orifice

Combination No.	Size of orifice.		Width of channel of approach.	Depth of water in channel of approach.	Bottom contraction.	Equation of discharge curve.	See text figure No.	Deviation from discharge table.			
	Depth.	Length.						Head.	Per cent.	Head.	Per cent.
	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>				<i>Ft.</i>		<i>Ft.</i>	
1	0.5	1.0	2.0	1.700	Without..	$Q = 0.604 a \sqrt{2g} H^{0.4969}$ .....	2	0.03	+ 3.9	0.75	0.0
2	.5	1.5	2.5	1.750	do.....	$Q = .606 a \sqrt{2g} H^{.4984}$ .....	2	.03	+ 3.7	.75	+ 0.5
3	.5	2.0	3.0	1.750	do.....	$Q = .606 a \sqrt{2g} H^{.4933}$ .....	2	.03	+ 2.3	.75	+ 0.4
4	.5	1.0	2.0	2.700	do.....	$Q = .602 a \sqrt{2g} H^{.5062}$ .....	2	.03	+ 0.5	.75	- 0.5
5	.5	1.5	2.5	2.750	do.....	$Q = .609 a \sqrt{2g} H^{.5094}$ .....	2	.03	- 1.4	.75	+ 0.6
6	.5	2.0	3.0	2.750	do.....	$Q = .605 a \sqrt{2g} H^{.4978}$ .....	2	.03	+ 0.5	.75	+ 0.1
7	1.0	2.0	3.0	2.500	do.....	$Q = .629 a \sqrt{2g} H^{.4987}$ .....	2	.03	+ 3.8	.75	+ 2.3
8	1.0	3.0	4.0	2.500	do.....	$Q = .631 a \sqrt{2g} H^{.5054}$ .....	2	.03	- 2.2	.75	+ 1.8
9	1.0	4.0	5.0	2.500	do.....	$Q = .627 a \sqrt{2g} H^{.4861}$ .....	2	.03	+ 0.6	.75	+ 1.1
10	.5	1.0	3.0	1.750	do.....	$Q = .602 a \sqrt{2g} H^{.4976}$ .....	2	.03	+ 3.2	.75	- 0.5
11	.5	1.5	3.5	1.750	do.....	$Q = .603 a \sqrt{2g} H^{.5040}$ .....	2	.03	- 0.5	.75	- 0.2
12	.5	2.0	4.0	1.750	do.....	$Q = .605 a \sqrt{2g} H^{.4978}$ .....	2	.03	+ 0.4	.75	0.0
13	.5	1.0	3.0	2.750	do.....	$Q = .605 a \sqrt{2g} H^{.5063}$ .....	2	.03	+ 0.7	.75	- 0.2
14	.5	1.5	3.5	2.750	do.....	$Q = .601 a \sqrt{2g} H^{.5017}$ .....	2	.03	0.0	.75	- 0.4
15	.5	2.0	4.0	2.750	do.....	$Q = .602 a \sqrt{2g} H^{.5027}$ .....	2	.03	- 1.8	.75	- 0.6
16	1.0	2.0	4.0	2.500	do.....	$Q = .614 a \sqrt{2g} H^{.5005}$ .....	2	.03	+ 0.8	.75	- 0.1
17	1.0	3.0	5.0	2.500	do.....	$Q = .611 a \sqrt{2g} H^{.4876}$ .....	2	.03	+ 0.7	.75	- 1.0
18	1.0	4.0	6.0	2.500	do.....	$Q = .617 a \sqrt{2g} H^{.4845}$ .....	2	.03	+ 0.2	.75	- 0.4
19	1.5	3.0	5.0	2.500	do.....	$Q = .644 a \sqrt{2g} H^{.4950}$ .....	2	.03	- 0.1	.50	+ 1.0
20	1.5	4.0	6.0	2.500	do.....	$Q = .639 a \sqrt{2g} H^{.4805}$ .....	2	.03	+ 0.1	.40	0.0
21	1.5	4.5	6.5	2.500	do.....	$Q = .638 a \sqrt{2g} H^{.4788}$ .....	2	.03	- 1.1	.35	- 0.7
22	2.0	4.0	6.0	2.500	do.....	$Q = .674 a \sqrt{2g} H^{.4762}$ .....	2	.03	+ 1.6	.25	+ 1.6
23	2.0	4.5	6.5	2.500	do.....	$Q = .692 a \sqrt{2g} H^{.4842}$ .....	2	.03	- 0.8	.20	+ 1.7
24	.5	1.0	4.0	1.750	do.....	$Q = .600 a \sqrt{2g} H^{.4967}$ .....	2	.03	+ 3.2	.75	- 0.8
25	.5	1.0	4.0	2.750	do.....	$Q = .599 a \sqrt{2g} H^{.4977}$ .....	2	.03	+ 2.7	.75	- 1.0
26	1.5	3.0	6.0	2.500	do.....	$Q = .623 a \sqrt{2g} H^{.4850}$ .....	2	.03	+ 0.1	.50	- 1.6
27	.5	1.0	2.0	1.625	With.....	$Q = .618 a \sqrt{2g} H^{.4975}$ .....	3	.03	+ 6.1	.75	+ 2.2
28	.5	1.5	2.5	1.600	do.....	$Q = .625 a \sqrt{2g} H^{.5005}$ .....	3	.03	+ 4.0	.75	+ 3.2
29	.5	2.0	3.0	1.600	do.....	$Q = .632 a \sqrt{2g} H^{.5005}$ .....	3	.03	+ 3.9	.75	+ 4.5
30	.5	1.0	2.0	2.625	do.....	$Q = .616 a \sqrt{2g} H^{.5067}$ .....	3	.03	+ 2.7	.75	+ 1.6
31	.5	1.5	2.5	2.600	do.....	$Q = .621 a \sqrt{2g} H^{.5008}$ .....	3	.03	+ 3.2	.75	+ 2.5
32	.5	2.0	3.0	2.600	do.....	$Q = .626 a \sqrt{2g} H^{.4981}$ .....	3	.03	+ 3.9	.75	+ 3.6
33	1.0	2.0	3.0	2.500	do.....	$Q = .642 a \sqrt{2g} H^{.4958}$ .....	3	.03	+ 7.1	.75	+ 4.5
34	1.0	3.0	4.0	2.500	do.....	$Q = .655 a \sqrt{2g} H^{.4964}$ .....	3	.03	+ 5.1	.75	+ 6.0
35	.5	1.5	3.5	2.600	do.....	$Q = .618 a \sqrt{2g} H^{.5019}$ .....	3	.03	+ 2.4	.75	+ 2.0
36	.5	2.0	4.0	2.600	do.....	$Q = .620 a \sqrt{2g} H^{.4987}$ .....	3	.03	+ 2.6	.75	+ 2.5
37	1.0	2.0	4.0	2.500	do.....	$Q = .632 a \sqrt{2g} H^{.4977}$ .....	3	.03	+ 4.7	.75	+ 2.8
38	1.0	3.0	5.0	2.500	do.....	$Q = .632 a \sqrt{2g} H^{.4922}$ .....	3	.03	+ 6.2	.75	+ 2.6
39	1.0	4.0	6.0	2.500	do.....	$Q = .635 a \sqrt{2g} H^{.4887}$ .....	3	.03	+ 1.7	.75	+ 2.5
40	1.5	3.0	5.0	2.500	do.....	$Q = .662 a \sqrt{2g} H^{.4948}$ .....	3	.03	+ 3.0	.50	+ 4.0
41	1.5	4.0	6.0	2.500	do.....	$Q = .656 a \sqrt{2g} H^{.4847}$ .....	3	.03	+ 1.1	.40	+ 2.1
42	1.5	4.5	6.5	2.500	do.....	$Q = .667 a \sqrt{2g} H^{.4860}$ .....	3	.03	+ 0.9	.35	+ 3.2
43	2.0	4.0	6.0	2.500	do.....	$Q = .697 a \sqrt{2g} H^{.4810}$ .....	3	.03	+ 3.2	.25	+ 4.3
44	2.0	4.5	6.5	2.500	do.....	$Q = .685 a \sqrt{2g} H^{.4707}$ .....	3	.03	+ 2.9	.20	+ 2.9
45	.5	1.5	3.5	1.600	do.....	$Q = .626 a \sqrt{2g} H^{.5022}$ .....	3	.03	+ 1.1	.75	+ 3.1
46	.5	2.0	4.0	1.600	do.....	$Q = .626 a \sqrt{2g} H^{.4970}$ .....	3	.03	+ 4.3	.75	+ 3.6
47	.5	1.0	4.0	1.625	do.....	$Q = .618 a \sqrt{2g} H^{.5028}$ .....	3	.03	+ 4.2	.75	+ 2.0
48	.5	1.0	4.0	2.625	do.....	$Q = .612 a \sqrt{2g} H^{.4922}$ .....	3	.03	+ 4.4	.75	+ 1.2
49	.5	2.0	10.0	2.600	do.....	$Q = .619 a \sqrt{2g} H^{.4993}$ .....	3	.03	+ 2.3	.75	+ 2.4
50	.5	2.0	10.0	2.600	do.....	$Q = .617 a \sqrt{2g} H^{.5020}$ .....	3	.03	+ 0.9	.75	+ 1.9



TABLE I.—Summary of results of experiments, showing the influence of various changes in the size of the box and the arrangement of the orifice—Continued

Combination No.	Size of orifice.		Width of channel of approach.	Depth of water in channel of approach.	Bottom contraction.	Equation of discharge curve.	See text figure No.	Deviation from discharge table.			
	Depth.	Length.						Head.	Per cent.	Head.	Per cent.
	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>	<i>Ft.</i>				<i>Ft.</i>		<i>Ft.</i>	
51	1.0	2.0	4.0	2.500	With.....	$Q=0.665 a \sqrt{2g} H^{0.5019}$ .....	4	0.03	+ 8.6	0.75	+ 8.1
52	1.0	2.0	4.0	2.500	...do.....	$Q=.637 a \sqrt{2g} H^{.4974}$ .....	5	.03	+ 5.6	.75	+ 3.6
53	1.0	2.0	4.0	2.500	...do.....	$Q=.694 a \sqrt{2g} H^{.4949}$ .....	6	.03	+16.0	.75	+13.0
54	1.0	2.0	4.0	2.500	...do.....	$Q=.694 a \sqrt{2g} H^{.4953}$ .....	7	.03	+15.7	.75	+12.8
55	1.0	2.0	4.0	2.500	...do.....	$Q=.643 a \sqrt{2g} H^{.4977}$ .....	8	.03	+ 6.3	.75	+ 4.4
56	1.0	2.0	4.0	2.500	...do.....	$Q=.669 a \sqrt{2g} H^{.5025}$ .....	9	.03	+ 8.9	.75	+ 8.6
57	1.5	4.0	6.0	2.500	...do.....	$Q=.674 a \sqrt{2g} H^{.4839}$ .....	4	.03	+ 4.4	.40	+ 5.2
58	1.5	4.0	6.0	2.500	...do.....	$Q=.669 a \sqrt{2g} H^{.4800}$ .....	10	.03	+ 5.0	.40	+ 4.8
59	1.5	4.0	6.0	2.500	...do.....	$Q=.659 a \sqrt{2g} H^{.4788}$ .....	5	.03	+ 3.9	.40	+ 3.3
60	1.5	4.0	6.0	2.500	...do.....	$Q=.722 a \sqrt{2g} H^{.4892}$ .....	6	.03	+ 9.6	.40	+11.9

Although constant care was used in making, setting, and calibrating the orifices, placing the sides and bottom of the orifice box, and observing precautions to eliminate all known sources of error, still there are a few inconsistencies, or what appear to be inconsistencies, in the data, though a more complete understanding of the flow through orifices of this type may show them to be due to more or less similar influences. Since the experimental data made straight-line logarithmic plots, only a few points were necessary to define those lines within a comparatively small percentage of error. Most of the curves for comparable conditions are practically parallel, but in two cases the curves cross. No. 8 in Table I crosses No. 17, and No. 5 crosses No. 14, these lines representing the data very faithfully.

The experimental conditions for the greater differences of head and for the longer orifices were less reliable than for the smaller discharges, but the general agreement of the data indicates that the accuracy was within practical demands at least. The arrangement of the control gate at the end of the channel of recession very probably produced a back-lash, which influenced the discharge, especially when the velocity of the water was great. The velocity of the water and cross currents also may have affected the hook-gage readings, but Table IV proves the average accuracy to be satisfactory. (See additional information given on p. 114.)

#### DEDUCTIONS FROM TABLE I

From an inspection of the coefficient and exponent values of the equations in Table I, the following general statements may be made:

The exponent decreases as the length of the orifice  $L$  increases so long as the depth of the orifice  $d$  and the cross-sectional area of the water in the channel of approach  $A$  remain constant. Although no two sets of experiments were made with exactly the same  $A$ , some are sufficiently close for purposes of comparison.

The exponent decreases as the ratio of the area of the orifice to the wetted cross-sectional area of the channel of approach  $\frac{a}{A}$  increases.

The coefficient increases with an increase in the depth of the orifice  $d$ .

The coefficient increases with an increase in the area of the orifice  $a$ , but does not increase regularly when the area of the orifice  $a$  has been divided out of the aggregate coefficient value.

Although the end-contraction distance has been taken as 1 foot for all sizes of orifices as a standard condition, a comparison of the deviation of discharges from the standard formula given in Table III indicates that little error would be introduced by making the contraction distance 0.5 foot for the smaller sizes of orifices. Increasing the distance to 1.5 feet would cause a greater error.

No. 13 to 23, inclusive, are for standard conditions. A comparison of these data with the data for other experiments, shows that lessening the depth of water in the channel of approach approximately 1 foot increases the discharge approximately 2 or 3 per cent. The increase is much greater for low heads than for high heads, especially with the smaller orifices. This action is difficult to explain, but it may be due to the existence of a critical velocity below which the velocity of approach has only a moderate effect upon the discharge and above which the effect may be somewhat overcome by increased friction and eddy currents.

The addition of an angle-iron bottom contraction and iron gate guides (No. 51) increases the discharge about 8 per cent.

No. 58 was an experiment to determine the effect upon the discharge caused by the iron gate guides projecting from the plane of the orifice as a comparatively narrow strip. A comparison of No. 58 and 57 shows that filling out the bulkhead until the face was flush with the edge of the gate guide (fig. 11) made practically no change in the discharge.

An orifice made of wood about  $1\frac{5}{8}$  inches thick, with a bottom contraction of the same material about  $1\frac{5}{8}$  inches high (No. 57), increased the discharge 3.3 per cent for the low head and 5.6 per cent for the high head. A comparison of this increase with that due to the angle-iron bottom contraction added to the standard condition of orifice indicates that the substitution of wood  $1\frac{5}{8}$  inches thick in place of the angle iron in the standard orifices will make the discharge about 2 per cent greater than that given in the standard discharge table. It will be observed that there would be no bottom contraction with this condition.

A wood orifice  $1\frac{5}{8}$  inches thick, with wood gate guides (fig. 7), will give a discharge from 9.6 to 16 per cent greater than that of the standard table, and the increase is the greatest for the smaller orifices (see No. 53 and 60). This increase probably is due to the guides being nearly the same distance apart as the length of the orifice, but set back from the plane of the orifice far enough to make the action similar to the flow through a short pipe.

No. 53 and 54 and figures 7 and 8 show the projection of the wood gate guides from the bulkhead to have little effect upon the discharge.

In No. 55 (fig. 9) the wood gate guides were set back a distance of 0.5 foot, and the resulting discharge was from 4.4 per cent to 6.3 per cent greater than the standard. A further comparison of the conditions shown in figures 7 and 9 indicate the discharge with the gate guides set back 0.5 foot to be only about 0.5 per cent greater than that for a plain wood orifice without any gate guides.

A wood orifice, with gate guides set back 0.5 foot and with a wood gate slide, as shown in No. 56 (fig. 10), gave a discharge about 8.6 to 8.9 per cent greater than the standard. A comparison of No. 56 with No. 55 indicates that the increase due to gate slide alone is from 3 to 4 per cent.

Complete end contractions on the upstream side of the orifice, and 0.5, foot end contractions on the downstream side, with a bottom contraction of  $1\frac{5}{8}$  inches (No. 49), gave a discharge from 2.3 to 2.4 per cent greater than that of the standard, and this was about the average increase due to the bottom angle-iron contraction with the standard orifice box. Therefore there was apparently little effect due to the complete end contraction in the channel of approach; but the decrease which, theoretically, should have resulted may have been counterbalanced by the increased velocity of approach caused by the smaller end contraction in the channel of recession.

Complete end contractions, both upstream and downstream from the orifice, but with an angle-iron bottom contraction of  $1\frac{5}{8}$  inches (No. 50), caused a deviation from the standard discharge of  $-0.5$  per cent for the low head and  $+2.9$  per cent for the high head. The discharge for the high head under this condition was therefore about the same as the standard size of orifice box with the angle-iron bottom contraction, but the increase in end contractions caused a decrease in the discharge for the low head of 2 or 3 per cent. The discharge curves represent the experimental data very accurately, and there is no apparent reason for the failure to decrease the discharge on the higher heads.

From the equations for No. 4 to 9 and 13 to 23, inclusive, it will be seen that, for a constant depth of water in the channel of approach, and for a constant depth of orifice, but for different lengths of orifices, where there are three lines in a set, the exponent value is the greatest for the middle length. A plot of the three points makes the curve apparent, even though the numerical values do not indicate it. The reverse of this curve is true for the coefficient values, as is shown by the several conditions of contraction, when comparable conditions are inspected. A similar comparison for No. 35 to 44, which are with bottom angle-iron contraction, shows both the coefficient and exponent value for the middle length to be the lowest. Therefore the insertion of the angle-iron bottom contraction seems to have reversed the curve for the law of the exponents, but produced no change in the curve for the coefficients.

# DERIVATION OF FORMULA FOR MODIFIED RECTANGULAR ORIFICES

Several unsuccessful attempts were made to derive a simple and accurate expression of the variation of the exponent and coefficient values in the individual equations No. 13 to 23, inclusive. As has been previously mentioned, the exponents and coefficients plot as a series of disconnected curves with the depths and lengths of orifices as the governing factors, apparently. The difficulty was experienced in determining just what factors should be used in an expression of the law of variation; and, though the following form is sufficiently accurate for practical purposes, it does not faithfully represent the variation. A more exact expression could have been obtained by using the same factors and expressing the variations as curves instead of straight lines, but the resulting formula would have been so complicated as to make it of doubtful practical value. It will be observed that the discharge formula appears to be more complicated than it really is, and the influence of velocity of approach has been expressed in terms of the wetted area of the channel of approach, which may be determined more easily, because there is a standard size of box for each length of orifice.

The experimental discharge data for the standard conditions of orifices and orifice boxes were plotted logarithmically against the areas of the orifices. The resulting series of curves were for each constant difference in head. From these curves the smoothed or balanced discharge values were taken and plotted logarithmically against the difference in heads. The equations of the average straight-line curves drawn through these points are given in Table II. The exponents in these equations were plotted against the ratio of the area of the orifice in square feet to the area of the wetted cross section of the channel of approach,  $\frac{a}{A}$ , as shown in figure 12. As previously noted, the exponents are in groups for the several areas of orifices with the same depth of orifice, and each group forms a detached curve which is probably parabolic in shape. To avoid a very complicated expression of their law of variation, they were assumed to be represented by straight lines which were parallel and had equal intercepts on the Y axis. The equation of each individual line is given in figure 12. The constants in these equations were plotted against the depths of the orifices (fig. 13), and the equation of the resulting curve was obtained. The substitution of this value in the equations given in figure 12 and with an average value for the coefficient of the ratio  $\frac{a}{A}$ , gave  $n = 0.4945 + 0.057d - \frac{0.257a}{A}$  as the general expression of the exponent of the head.

TABLE II.—Equations of balanced discharge curves used in development of general formula

Size of orifice.		Equation.
Depth.	Length.	
<i>Feet.</i>	<i>Feet.</i>	
0.5	1.0	$Q=2.424h^{0.5054}$
.5	1.5	$Q=3.624h^{0.5033}$
.5	2.0	$Q=4.822h^{0.4982}$
1.0	2.0	$Q=9.858h^{0.5013}$
1.0	3.0	$Q=14.842h^{0.4910}$
1.0	4.0	$Q=19.779h^{0.4822}$
1.5	3.0	$Q=22.751h^{0.4860}$
1.5	4.0	$Q=30.374h^{0.4780}$
1.5	4.5	$Q=34.293h^{0.4680}$
2.0	4.0	$Q=44.668h^{0.4840}$
2.0	4.5	$Q=48.600h^{0.4680}$

The coefficients of the revised equations given in Table II were plotted logarithmically against the areas of the orifices, and they also were in separate groups for each depth of orifice, but are not shown here because the reduction in the size of the plot would obscure the grouping. Straight lines, drawn to meet at a common point, fairly represent the several sets of plotted points, and give a simple law of their variation. The equations of these lines follow:

$$\text{When } d=0.5 \text{ foot, } c=4.85a^{1.00}.$$

$$d=1.0 \text{ foot, } c=4.90a^{1.01}.$$

$$d=1.5 \text{ feet, } c=4.95a^{1.02}.$$

$$d=2.0 \text{ feet, } c=5.00a^{1.03}.$$

The coefficient and exponent values of the area of the orifice,  $a$ , were plotted and found to be represented by  $(4.8+0.1d)$  and  $(0.02d+0.99)$ , respectively, which unite as the coefficient of the head  $c=(4.8+0.1d)a^{(0.02d+0.99)}$ .

Consolidating the expressions for the exponent and coefficient values of the head,  $h$ , gives the general formula for the discharge through submerged rectangular orifices placed according to the conditions which have been taken as the standard:

$$Q = ((4.8+0.1d)a^{(0.02d+0.99)})h^{(0.495d+0.06d-\frac{7.36a}{A})}$$

in which " $Q$ " equals the discharge in second-feet; " $d$ " equals depth of orifice in feet; " $a$ " equals area of orifice in square feet; " $A$ " equals area of cross section of water in channel of approach in square feet; and " $h$ " equals the difference in feet between the water levels upstream and downstream from the orifice.



TABLE III.—Discharges through modified submerged rectangular orifices as computed from the formula

Head in feet.	Head in inches.	Discharge (cubic feet per second).										
		0.5X1.0	0.5X1.5	0.5X2.0	1.0X2.0	1.0X3.0	1.0X4.0	1.5X3.0	1.5X4.0	1.5X4.5	3.0X4.0	2.0X4.5
0.03	0 3/8	0.408	0.624	0.843	1.69	2.64	3.61	4.10	5.70	6.52	8.02	9.22
0.04	0 1/2	.473	.721	.973	1.95	3.04	4.15	4.73	6.55	7.48	9.20	10.55
0.05	0 5/8	.529	.806	1.09	2.19	3.39	4.63	5.27	7.29	8.32	10.23	11.72
0.06	0 3/4	.581	.884	1.19	2.40	3.71	5.06	5.77	7.95	9.07	11.10	12.77
0.07	0 7/8	.628	.955	1.29	2.59	4.01	5.46	6.22	8.57	9.76	12.01	13.74
0.08	0 1	.672	1.02	1.38	2.77	4.28	5.82	6.64	9.14	10.41	12.79	14.63
0.09	1 1/8	.714	1.08	1.46	2.94	4.53	6.17	7.04	9.67	11.01	13.53	15.46
0.10	1 1/4	.753	1.14	1.54	3.10	4.78	6.49	7.41	10.17	11.57	14.23	16.25
0.11	1 1/2	.790	1.20	1.61	3.25	5.01	6.80	7.77	10.65	12.11	14.89	17.00
0.12	1 5/8	.826	1.25	1.68	3.40	5.23	7.09	8.11	11.10	12.63	15.52	17.71
0.13	1 3/4	.860	1.30	1.75	3.54	5.44	7.37	8.43	11.54	13.12	16.12	18.39
0.14	1 7/8	.893	1.35	1.82	3.67	5.64	7.64	8.74	11.96	13.59	16.70	19.04
0.15	1 1	.925	1.40	1.88	3.80	5.83	7.90	9.04	12.36	14.04	17.26	19.67
0.16	1 1/2	.956	1.45	1.94	3.93	6.02	8.16	9.33	12.75	14.48	17.80	20.28
0.17	2 1/8	.986	1.49	2.00	4.05	6.20	8.40	9.62	13.13	14.91	18.32	20.86
0.18	2 1/4	1.01	1.54	2.06	4.17	6.38	8.64	9.89	13.49	15.32	18.82	21.43
0.19	2 3/8	1.04	1.58	2.12	4.28	6.55	8.87	10.16	13.85	15.72	19.31	21.98
0.20	2 1/2	1.07	1.62	2.17	4.39	6.72	9.09	10.42	14.19	16.11	19.79	22.52
0.21	2 3/4	1.10	1.66	2.23	4.50	6.89	9.31	10.67	14.53	16.49	20.26	.....
0.22	2 5/8	1.12	1.70	2.28	4.61	7.04	9.52	10.92	14.86	16.86	20.71	.....
0.23	2 3/2	1.15	1.74	2.33	4.71	7.20	9.73	11.16	15.18	17.22	21.15	.....
0.24	2 7/8	1.17	1.77	2.38	4.81	7.35	9.93	11.39	15.50	17.57	21.58	.....
0.25	3 0	1.20	1.81	2.43	4.91	7.50	10.13	11.62	15.80	17.92	22.01	.....
0.26	3 1/8	1.22	1.85	2.48	5.01	7.65	10.33	11.85	16.10	18.26	.....	.....
0.27	3 1/4	1.25	1.88	2.52	5.11	7.79	10.52	12.07	16.40	18.59	.....	.....
0.28	3 3/8	1.27	1.92	2.57	5.20	7.93	10.71	12.29	16.69	18.91	.....	.....
0.29	3 1/2	1.29	1.95	2.62	5.29	8.07	10.89	12.50	16.97	19.23	.....	.....
0.30	3 3/4	1.32	1.99	2.66	5.38	8.21	11.07	12.71	17.25	19.55	.....	.....
0.31	3 3/2	1.34	2.02	2.70	5.47	8.34	11.25	12.92	17.53	19.86	.....	.....
0.32	3 7/8	1.36	2.05	2.75	5.56	8.47	11.42	13.12	17.80	20.16	.....	.....
0.33	3 1	1.38	2.08	2.79	5.65	8.61	11.59	13.32	18.06	20.46	.....	.....
0.34	4 1/8	1.40	2.11	2.83	5.74	8.73	11.76	13.52	18.32	20.75	.....	.....
0.35	4 1/4	1.42	2.15	2.87	5.82	8.86	11.93	13.71	18.58	21.04	.....	.....
0.36	4 1/2	1.44	2.18	2.91	5.90	8.98	12.09	13.90	18.83	.....	.....	.....
0.37	4 3/8	1.46	2.21	2.95	5.98	9.10	12.26	14.09	19.08	.....	.....	.....
0.38	4 1/2	1.48	2.24	2.99	6.07	9.22	12.42	14.27	19.33	.....	.....	.....
0.39	4 5/8	1.50	2.27	3.03	6.14	9.34	12.58	14.46	19.57	.....	.....	.....
0.40	4 1/2	1.52	2.29	3.07	6.22	9.46	12.73	14.64	19.81	.....	.....	.....
0.41	4 3/4	1.54	2.32	3.11	6.30	9.58	12.88	14.82	.....	.....	.....	.....
0.42	5 1/8	1.56	2.35	3.15	6.38	9.69	13.04	14.99	.....	.....	.....	.....
0.43	5 1/4	1.58	2.38	3.18	6.45	9.80	13.19	15.17	.....	.....	.....	.....
0.44	5 3/8	1.60	2.41	3.22	6.53	9.91	13.33	15.34	.....	.....	.....	.....
0.45	5 1/2	1.62	2.43	3.26	6.60	10.03	13.48	15.51	.....	.....	.....	.....
0.46	5 3/4	1.63	2.46	3.29	6.68	10.13	13.62	15.68	.....	.....	.....	.....
0.47	5 5/8	1.65	2.49	3.33	6.75	10.24	13.77	15.84	.....	.....	.....	.....
0.48	5 3/2	1.67	2.51	3.36	6.82	10.35	13.91	16.01	.....	.....	.....	.....
0.49	5 7/8	1.69	2.54	3.40	6.89	10.46	14.05	16.17	.....	.....	.....	.....
0.50	6 0	1.71	2.57	3.43	6.96	10.56	14.19	16.33	.....	.....	.....	.....
0.51	6 1/8	1.72	2.59	3.47	7.03	10.66	14.33	.....	.....	.....	.....	.....
0.52	6 1/4	1.74	2.62	3.50	7.10	10.77	14.46	.....	.....	.....	.....	.....
0.53	6 3/8	1.76	2.64	3.53	7.17	10.87	14.60	.....	.....	.....	.....	.....
0.54	6 1/2	1.77	2.67	3.57	7.24	10.97	14.73	.....	.....	.....	.....	.....
0.55	6 5/8	1.79	2.69	3.60	7.31	11.07	14.86	.....	.....	.....	.....	.....
0.56	6 3/4	1.81	2.72	3.63	7.37	11.17	14.99	.....	.....	.....	.....	.....
0.57	6 7/8	1.82	2.74	3.66	7.44	11.27	15.12	.....	.....	.....	.....	.....
0.58	6 1	1.84	2.77	3.70	7.50	11.36	15.25	.....	.....	.....	.....	.....
0.59	7 1/8	1.85	2.79	3.73	7.57	11.46	15.38	.....	.....	.....	.....	.....
0.60	7 1/4	1.87	2.81	3.76	7.63	11.55	15.50	.....	.....	.....	.....	.....
0.61	7 3/8	1.89	2.84	3.79	7.70	11.65	15.63	.....	.....	.....	.....	.....
0.62	7 1/2	1.90	2.86	3.82	7.76	11.74	15.75	.....	.....	.....	.....	.....
0.63	7 5/8	1.92	2.88	3.85	7.82	11.83	15.88	.....	.....	.....	.....	.....
0.64	7 3/4	1.93	2.91	3.88	7.88	11.93	16.00	.....	.....	.....	.....	.....
0.65	7 7/8	1.95	2.93	3.91	7.95	12.02	16.12	.....	.....	.....	.....	.....
0.66	8 1/8	1.96	2.95	3.94	8.01	12.11	16.24	.....	.....	.....	.....	.....
0.67	8 1/4	1.98	2.97	3.97	8.07	12.20	16.36	.....	.....	.....	.....	.....
0.68	8 3/8	1.99	3.00	4.00	8.13	12.29	16.48	.....	.....	.....	.....	.....
0.69	8 1/2	2.01	3.02	4.03	8.19	12.38	16.59	.....	.....	.....	.....	.....
0.70	8 3/4	2.02	3.04	4.06	8.25	12.46	16.71	.....	.....	.....	.....	.....
0.71	8 5/8	2.04	3.06	4.09	8.31	12.55	16.83	.....	.....	.....	.....	.....
0.72	8 3/2	2.05	3.08	4.12	8.37	12.64	16.94	.....	.....	.....	.....	.....
0.73	8 3/4	2.07	3.11	4.15	8.42	12.73	17.06	.....	.....	.....	.....	.....
0.74	8 7/8	2.08	3.13	4.17	8.48	12.81	17.17	.....	.....	.....	.....	.....
0.75	9 0	2.10	3.15	4.20	8.54	12.90	17.28	.....	.....	.....	.....	.....

The agreement of the discharge formula with the experimental data is shown in Table IV to be within a mean of approximately 0.5 per cent, but there are a few individual points more than 1 per cent off.

TABLE IV.—Difference between discharge obtained by experiment and as computed from the formula

Size of orifice.	Difference of heads.	Discharge (cubic feet per second).			Experimental discharge compared with curve.		Computed discharge compared with curve.	
		From experiment.	From average curve.	Computed from formula.	Difference (cubic feet per second).	Per cent.	Difference (cubic feet per second).	Per cent.
<i>Square feet.</i>	<i>Feet.</i>							
0.5 by 1.0 foot (exact area, 0.4997 square foot).....	0.1	0.756	0.756	0.751	0.000	0.00	-0.005	-0.66
	.4	1.525	1.525	1.521	0.000	0.00	-0.004	-0.26
	.6	1.876	1.873	1.869	+0.003	+0.16	-0.004	-0.21
	.8	2.163	2.167	2.164	-0.004	-0.18	-0.003	-0.14
0.5 by 1.5 feet (exact area, 0.7521 square foot).....	.1	1.141	1.141	1.140	0.000	0.00	-0.001	-0.09
	.3	1.987	1.983	1.986	+0.005	+0.25	+0.004	+0.20
	.5	2.548	2.559	2.571	-0.011	-0.43	+0.012	+0.47
	.8	3.239	3.237	3.259	+0.002	+0.06	+0.022	+0.68
0.5 by 2.0 feet (exact area, 0.9996 square foot).....	.1	1.511	1.517	1.530	-0.006	-0.40	+0.013	+0.86
	.3	2.636	2.638	2.652	-0.002	-0.08	+0.014	+0.53
	.5	3.404	3.407	3.426	-0.003	-0.09	+0.019	+0.56
	.8	4.318	4.314	4.335	+0.004	+0.09	+0.021	+0.49
1.0 by 2.0 feet (exact area, 2.0001 square feet).....	.1	3.108	3.114	3.100	-0.006	-0.18	-0.014	-0.45
	.3	5.407	5.398	5.386	+0.009	+0.17	-0.012	-0.22
	.5	6.969	6.966	6.964	+0.003	+0.04	-0.002	-0.03
	.8	8.814	8.815	8.821	-0.001	-0.01	+0.006	+0.07
1.0 by 3.0 feet (exact area, 2.9997 square feet).....	.1	4.785	4.781	4.776	+0.004	+0.08	-0.005	-0.10
	.3	8.158	8.170	8.209	-0.012	-0.15	+0.039	+0.48
	.5	10.471	10.476	10.559	-0.005	-0.05	+0.083	+0.79
	.8	13.186	13.186	13.312	0.000	0.00	+0.126	+0.96
1.0 by 4.0 feet (exact area, 3.9970 square feet).....	.1	6.505	6.471	6.486	+0.034	+0.52	+0.015	+0.23
	.2	8.983	9.062	9.085	-0.079	-0.97	+0.023	+0.25
	.4	12.682	12.688	12.722	-0.006	-0.05	+0.034	+0.27
	.7	16.603	16.661	16.699	-0.058	-0.35	+0.038	+0.23
1.5 by 3.0 feet (exact area, 4.4998 square feet).....	.1	7.428	7.430	7.411	-0.002	-0.03	-0.019	-0.26
	.2	10.507	10.471	10.414	+0.036	+0.34	-0.057	-0.54
	.4	14.888	14.750	14.638	+0.138	+0.94	-0.112	-0.76
	.5	16.488	16.474	16.332	+0.014	+0.09	-0.142	-0.86
1.5 by 4.0 feet (exact area, 6.0006 square feet).....	.6	18.103	18.030	17.863	+0.073	+0.40	-0.167	-0.93
	.10	10.155	10.170	10.172	-0.015	-0.15	+0.002	+0.02
	.15	12.435	12.365	12.361	+0.070	+0.57	-0.004	-0.03
	.20	14.275	14.181	14.196	+0.094	+0.66	+0.015	+0.11
1.5 by 4.5 feet (exact area, 6.7513 square feet).....	.25	15.741	15.813	15.803	-0.072	-0.45	-0.010	-0.06
	.30	17.313	17.258	17.253	+0.055	+0.32	-0.005	-0.03
	.339	18.398	18.302	18.297	+0.096	+0.52	-0.005	-0.03
	.363	18.919	18.915	18.909	+0.004	+0.02	-0.006	-0.03
1.5 by 4.5 feet (exact area, 6.7513 square feet).....	.1	11.356	11.479	11.576	-0.123	-1.07	+0.097	+0.85
	.2	15.978	15.993	16.114	-0.015	-0.09	+0.121	+0.76
	.25	17.865	17.791	17.922	+0.074	+0.42	+0.131	+0.74
	.283	18.818	18.880	19.016	-0.062	-0.33	+0.136	+0.72
2.0 by 4.0 feet (exact area, 8.0065 square feet).....	.05	10.512	10.407	10.239	+0.105	+1.01	-0.168	-1.61
	.10	14.498	14.481	14.241	+0.017	+0.12	-0.240	-1.66
	.10	14.398	14.481	14.241	-0.083	-0.57	-0.240	-1.66
	.15	17.671	17.571	17.270	+0.100	+0.57	-0.301	-1.71
2.0 by 4.5 feet (exact area, 8.9990 square feet).....	.15	17.810	17.571	17.270	+0.239	+1.36	-0.301	-1.71
	.05	11.710	11.714	11.722	-0.004	-0.03	+0.008	+0.07
	.10	16.368	16.379	16.249	-0.011	-0.07	-0.130	-0.79
	.125	18.276	18.260	18.047	+0.016	+0.09	-0.213	-1.17

The computed discharges agree with the discharges taken from the experimental curves within a maximum error of less than 1 per cent, except on the 2 by 4 foot and the 2 by 4.5 foot orifices, where the experimental control was not entirely dependable and somewhat in error.

The computed discharges agree with the experimental discharge data within 1 per cent, except for the large orifices noted above. It is therefore safe to assume that this type of orifice and the discharge formula for it will give results within 2 per cent of the truth for all cases, and probably within 1 per cent for the majority of cases.

Notwithstanding the fact that this type of orifice will permit a measurement of flow with an accuracy well within practical demands and has other previously enumerated practical advantages, it must be borne in mind that a correction factor will have to be applied to the tables unless the depth of water in the channel of approach is 2.5 feet, which is the depth upon which the formula and tables are based. Such correction factors are not only bothersome, but often are a source of grave error; and therefore it is desirable to maintain the standard depth of water if possible. The correction factor is made necessary for a change in depth of water in the channel of approach because of a changed velocity of approach and also because of a changed contraction distance at the top of the orifice. Where the standard depth of water, 2.5 feet, can not be obtained, or where there is a considerable fluctuation in the depth of water, the use of the modified submerged orifice should be discouraged.

#### SUPPLEMENTAL TESTS ON SUBMERGED ORIFICES

Some unusual results were obtained from the orifice experiments made in the hydraulic laboratory in the summer of 1914, and because of the revolutionary character of those original data, special care has been taken to insure their accuracy. Every part of the apparatus and every phase of the results that offered a very probable source of error have been questioned and examined. The data were consistent with themselves, but did not conform to the somewhat arbitrary theory that has grown piecemeal from isolated parts of experiments. It was thought probable that the gate placed at the end of the channel of recession to control the submergence on the orifice was so close to the orifice as to have a marked effect upon the flow through the orifice.

A series of control experiments was made during the summer of 1916, in which the orifice structures were duplicates of those used in the original experiments, the heads were the same, the same general methods of experimentation were used; but the 1916 check experiments were made in the concrete rating channel outside the laboratory. The orifice structure was placed near the middle of the channel, which is 200 feet long, 5 feet wide, and 3.5 feet deep, and there is no gate or other obstruction in the channel within approximately 100 feet of the orifice. Although it

was not possible to control the flow of water as perfectly in the long channel as in the laboratory proper, still the regulation of the head was quite good, and in all experiments the discharge was determined volumetrically.

Of the 22 experiments made for check purposes, 15 were comparable directly with original experiments made in 1914. They show that the gate caused an increase in the discharge, but, when allowance is made for probable experimental error, this increase is a maximum of approximately 2 per cent. The 1916 experimental results do not show the original data to have been in error an impractical amount due to the close proximity of the control gate to the orifice.

# THE VENTURI FLUME

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## INTRODUCTION

Many devices have been developed for the measurement of water under field conditions—for example, in its delivery to irrigators. Nearly all of these devices employ the principles of either the weir or the orifice and, though each device is adapted to use in certain localities, probably none works satisfactorily under a great variety of field conditions. The ideal measuring device would (1) be inexpensive to construct, (2) be simple to operate, (3) require little maintenance, (4) be free from working parts, (5) be accurate in its measurement, (6) be free from sand, silt, or floating-trash troubles, and (7) require but little loss of head in the ditch. Such a panacea for all measurement-of-water ills does not seem probable, but progress is undoubtedly being made toward that end. The type of flume tested in the experiments on which this report is based possesses many of the qualities enumerated and may prove to be a satisfactory measuring device under general field conditions.

The purpose of this article is to present the fundamental plans and results of preliminary experiments on a new type of device, called the "Venturi flume," for measuring water in open channels, in order that those in practical need of such a device may know of its existence. Furthermore, it is hoped that the construction of larger sizes of Venturi flumes than were tested in the laboratory will be encouraged thereby and that they can be calibrated. It is not probable that the last word has been said on the design of the Venturi flume, for, although it has considerable promise, changes in details may prove to be necessary. The laboratory and field tests made thus far have failed to develop any serious inherent defects in the device.

Experiments made in the hydraulic laboratory at Fort Collins, Colorado, on measuring devices led to the development of the so-called Venturi flume during the season of 1915. It consists essentially of a flume with a converging and a diverging section and short "throat" section between them. The floor, which is level, is placed at the elevation of the bottom of the channel in which it is set. After many experiments had been made with different forms and shapes, the designs shown in figures 1, 6, 7, and 11 were adopted as most nearly meeting practical requirements. Venturi flumes with rectangular and trapezoidal cross sections (fig. 1, 6) no doubt will be the most used, but the other types (fig. 7, 11) were designed to meet special conditions where small flows must be measured.



The action of this device depends upon an adaptation or extension of Venturi's principle to the flow of a liquid in an open channel. As water passes through the flume there is a slight surface slope in the converging section, a rather sudden depression in the "throat" section, and a rise in the diverging section. The actual loss of head is small. The determination of the flow depends upon the velocity and wetted cross-sectional area at two points in the flume, and two gage readings, therefore, are necessary. One gage has been arbitrarily located upstream from the throat a distance equal to two-thirds the length of the converging section, to avoid possible influence due to contraction currents nearer the entrance to the flume; and the other gage has been located at the middle of the throat section, in order to obtain the greatest possible difference in elevation of water surface. The zero of these gages must be at the elevation of the floor of the flume, and it is especially important that the zero of the gages be at exactly the same elevation. The difference in heads,  $H_a$ , is a more important factor in determining the discharge than the depth of water in the channel,  $H_a$  or  $H_b$ .

Still boxes, or gage wells, are necessary for accurate readings of the water levels, because of the comparatively high velocity of the water flowing through the structure. Field tests on small Venturi flumes<sup>1</sup> indicated that readings taken to the nearest 0.01 foot on staff gages placed at the proper locations inside the flume, with the face of the gages countersunk flush with the surface of the side of the flume, would give an accuracy of measurement sufficient for general purposes. This would overcome the necessity for using gage wells, but recent tests made in the laboratory show that such staff-gage readings do not agree with readings taken in the gage wells when there is enough fall in the carrying channel to give a high velocity of flow through the flume, in which case  $H_a$  is a considerable amount. Until more is known of the accuracy of gages under different arrangements, caution should be used.

Instrument makers are at work on an automatic register to make graphs of the water elevations at the two gages, both records to appear on a single sheet. An integrating register would be most desirable, but the complexity of the law of flow through the flume certainly would require a complicated instrument.

The effect of the velocity of approach is automatically cared for in the device, and the formula takes account of the velocity of the water at each gage. The experiments indicate that the Venturi flume will be free from interference due to changes in the canal section, such as occur often from sand or silt accumulations or aquatic growths. Such obstructions make the use of the ordinary rating flume very troublesome, if not quite impossible, but these obstructions result only in changing the relative gage readings of the Venturi flume without altering the calibration of

<sup>1</sup> Tests made on the North Platte Project, United States Reclamation Service, Mitchell, Nebraska, under the general direction of Mr. Andrew Weiss, Project Manager.

the device. Since the velocity increases throughout the converging section, all material carried into the flume also will be carried out, and this self-cleaning feature is of considerable practical importance. When the depth of water is low, floating trash might lodge in the throat of the V-notch Venturi flume, which is of small cross section, but it would cause an accumulation of water in the upstream channel until the wetted cross section at the throat would be sufficient to allow the obstruction to pass. It must be borne in mind that a Venturi flume of whatever form must not be placed below canal grade, for this would give a standing-water condition which would alter the calibration of the device, and it would also allow sand and silt to accumulate within the structure at low velocities. It is important also that the width of the channel of approach be not greatly in excess of the greatest width of the flume, as this permits a silt bank to be deposited at either side wing of the flume.

A desirable phase of this device is the practical connection which it may make with the ditch banks. At the ends of the structure, wings may be placed at an angle of  $90^\circ$  to the axis of the structure to make the connection with the ditch banks, or the ends of the structure may be joined directly to the ditch lining.

Another practical feature in connection with the Venturi flume is the small loss of head required for purposes of measuring the flow. Table I shows for the V-notch flume the lost head for the different discharges obtained with different depths of water. The head at the upstream gage is called  $H_a$ , the head at the throat gage is called  $H_b$ , and the difference between these heads ( $H_a - H_b$ ) is called  $H_d$ . Under usual conditions of operation the lost head will be negligible.

TABLE I.—Loss in head (in feet) in V-notch Venturi flume for different heads at the two gages

$H_a$	$H_d=0.05$		$H_d=0.10$		$H_d=0.15$		$H_d=0.20$		$H_d=0.25$		$H_d=0.30$	
	Q in sec.-feet.	Loss in head in feet.	Q in sec.-feet.	Loss in head in feet.	Q in sec.-feet.	Loss in head in feet.	Q in sec.-feet.	Loss in head in feet.	Q in sec.-feet.	Loss in head in feet.	Q in sec.-feet.	Loss in head in feet.
0.4.....	0.10	0.06	0.11	0.08	.....	.....	.....	.....	.....	.....	.....	.....
.6.....	.26	.05	.30	.07	.....	.....	.....	.....	.....	.....	.....	.....
.8.....	.49	.04	.60	.06	0.62	0.15	.....	.....	.....	.....	.....	.....
1.0.....	.81	.03	1.00	.06	1.09	.13	1.12	0.24	.....	.....	.....	.....
1.2.....	1.20	.03	1.52	.06	1.63	.11	1.76	.17	1.79	0.29	.....	.....
1.4.....	1.71	.03	2.17	.05	2.42	.10	2.56	.14	2.63	.21	2.67	0.31
1.6.....	2.33	.03	2.96	.05	3.31	.09	3.52	.12	3.65	.17	3.74	.23

#### RECTANGULAR VENTURI FLUME

The original idea was to invent a device which would replace the ordinary rating flume, such as is used in irrigation canals. It was thought that the flume might be converted into a self-contained measuring device by placing a restricted section in the flume, which would cause a loss of

head, and a determination of such loss of head would indicate the volume of water flowing in the channel. Thus far, Venturi's principle had not been considered in the action of such a device. Small flumes with vertical sides were used in the preliminary experiments; and, after employing several different ratios of widths of throat to lengths of flume, lengths

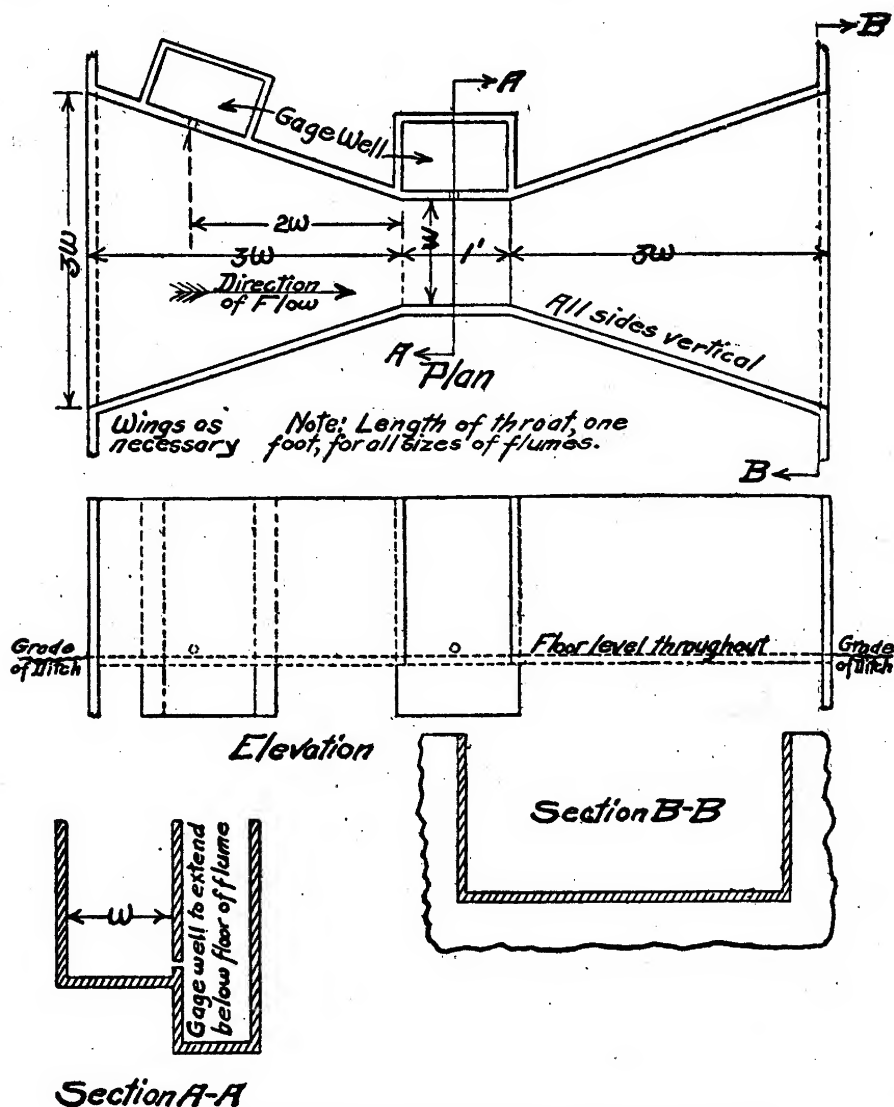
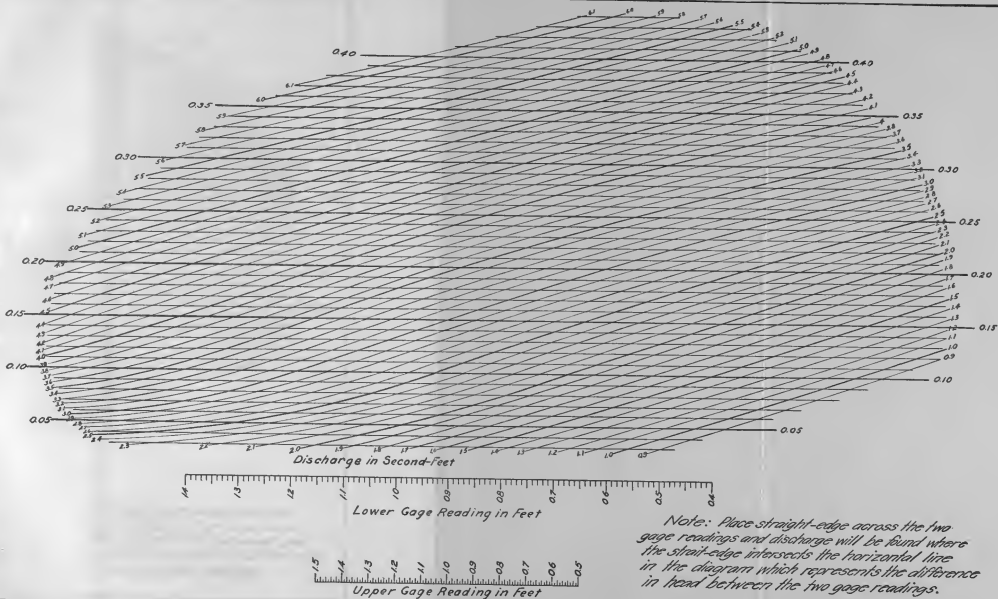
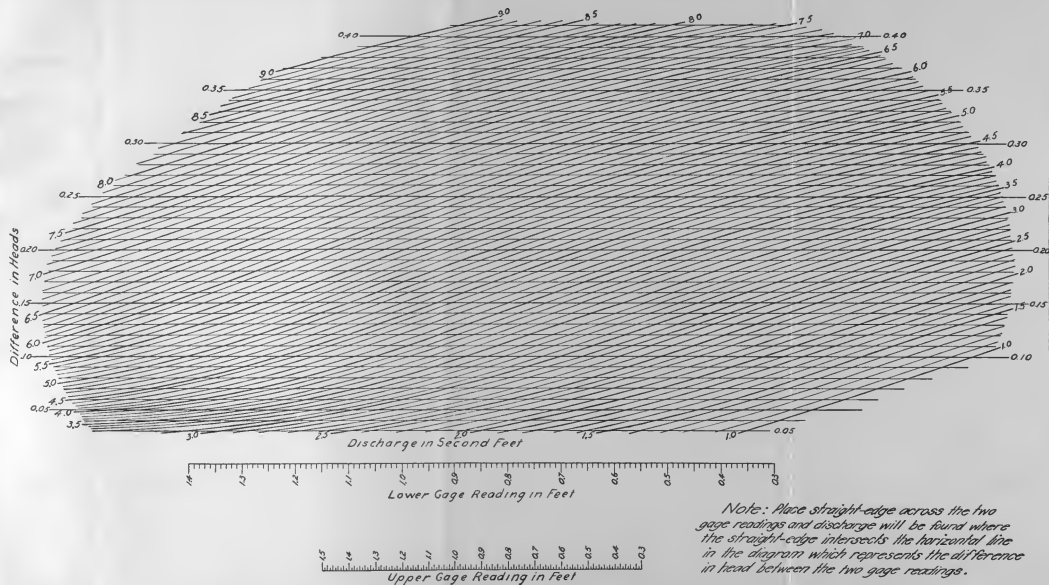


FIG. 1.—Standard plans for the Venturi flume with rectangular cross section.

of throat, and arrangements of gages and end wings, the form shown in figure 1 was chosen as the standard. A greater length of converging and diverging section and a rounding of the throat section would result in less loss of head and greater accuracy in measurement of flow, but the standard was chosen as a compromise between accuracy and cost.







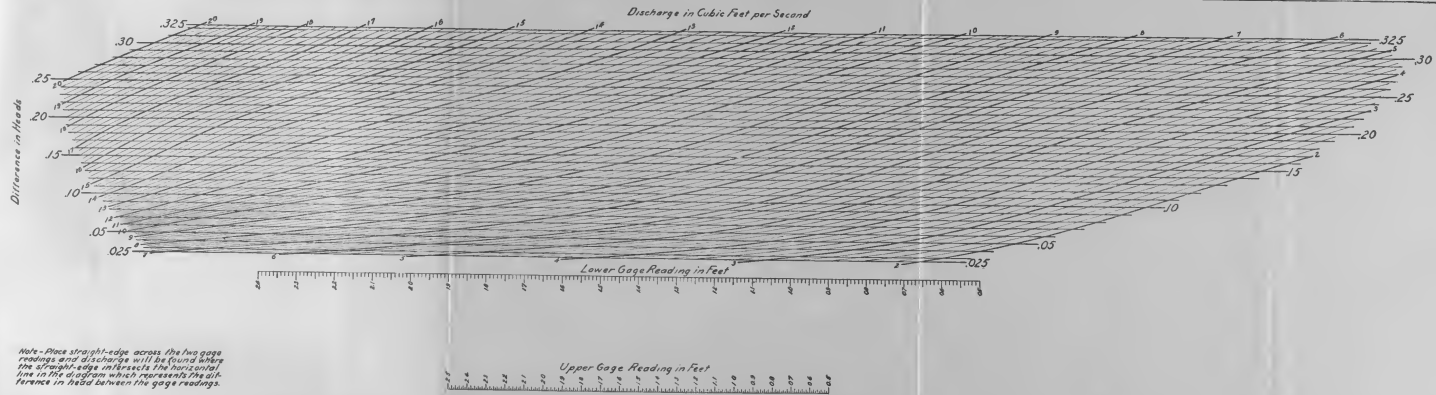
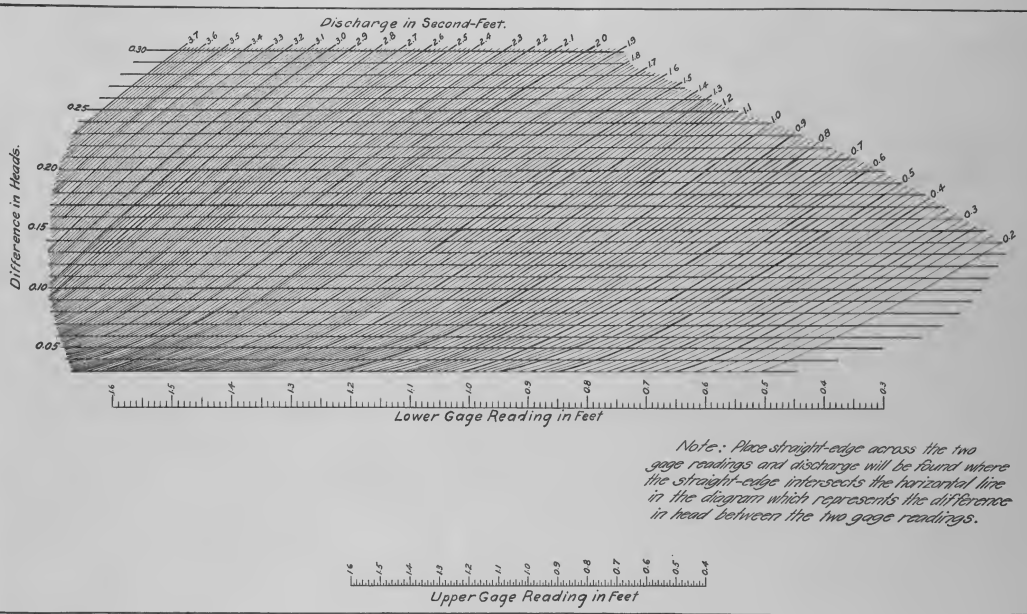


FIG. 4.—Discharge curves for the rectangular Venturi flume with 2-foot throat.



$$1 = \frac{H_a}{H_b^4} - \frac{H_d}{(2.75 + H_a)^2 H_a^2}$$

$H_a$  = Head at upstream gage.  
 $H_b$  = Head at throat gage.  
 $H_d$  = Difference in gage readings ( $H_a - H_b$ ).

The Venturi flume with rectangular cross section is especially simple to build of any material suitable for use in water, and will probably be the most popular type. Its practical minimum throat width is 1 foot, and the largest one thus far constructed has a throat width of 7 feet.

A general formula for the discharge through rectangular Venturi flumes has not been worked out, because calibrations have not been made on flumes large enough to warrant a formula of general application. Discharge curves are given in figures 2, 3, and 4 for throat widths of 1,  $1\frac{1}{2}$ , and 2 feet.

#### TRAPEZOIDAL VENTURI FLUME WITH SIDE SLOPES OF $1\frac{1}{2}$ TO 1

Although no trapezoidal Venturi flumes with side slopes of  $1\frac{1}{2}$  to 1 have been constructed, there is no reason apparent why their behavior would not be similar to that of rectangular cross-sectional type. The side slopes of  $1\frac{1}{2}$  to 1 will fit the majority of canal banks, and the resulting cross section will accommodate a greater range of discharges than the rectangular flumes. Therefore it is believed that, for the larger canals, the more satisfactory type of Venturi flume will have a trapezoidal cross section with side slopes of  $1\frac{1}{2}$  to 1. It will fit nicely with concrete lining of canals. This form does not call for warped surfaces, because the slopes are taken normal to the axial line of the flume, which is in a plane normal to the side of the throat section but is not normal to the side of the converging and diverging sections. The general plans for this type are given in figure 6, but no discharge curves are available at this time. It is expected that calibrations will be made from structures as they are installed under actual field conditions.

#### V-NOTCH VENTURI FLUME

There has been a demand for many years for a device to measure small flows of water where the permissible loss of head is small, or where sand and silt is carried by the water. After repeated unsuccessful attempts had been made to arrange a modification of an orifice or weir to meet these conditions, it was decided to ascertain what combination could be made of the Venturi flume and the triangular-notch weir. The result was the V-notch Venturi flume shown in figures 7 and 8. The side slopes of  $\frac{1}{2}$  to 1, in a plane normal to the axis of the flume, give a cross-sectional area of the throat section for different depths of water, which allows a good range of discharge from extreme high to low heads. This form is applicable under conditions of head commonly found in small ditches to flows of from 0.1 to 2 or 3 second-feet.

Discharges through V-notch Venturi flumes are given in graphic form in figure 5, and those computed from the formula are given in Table II.

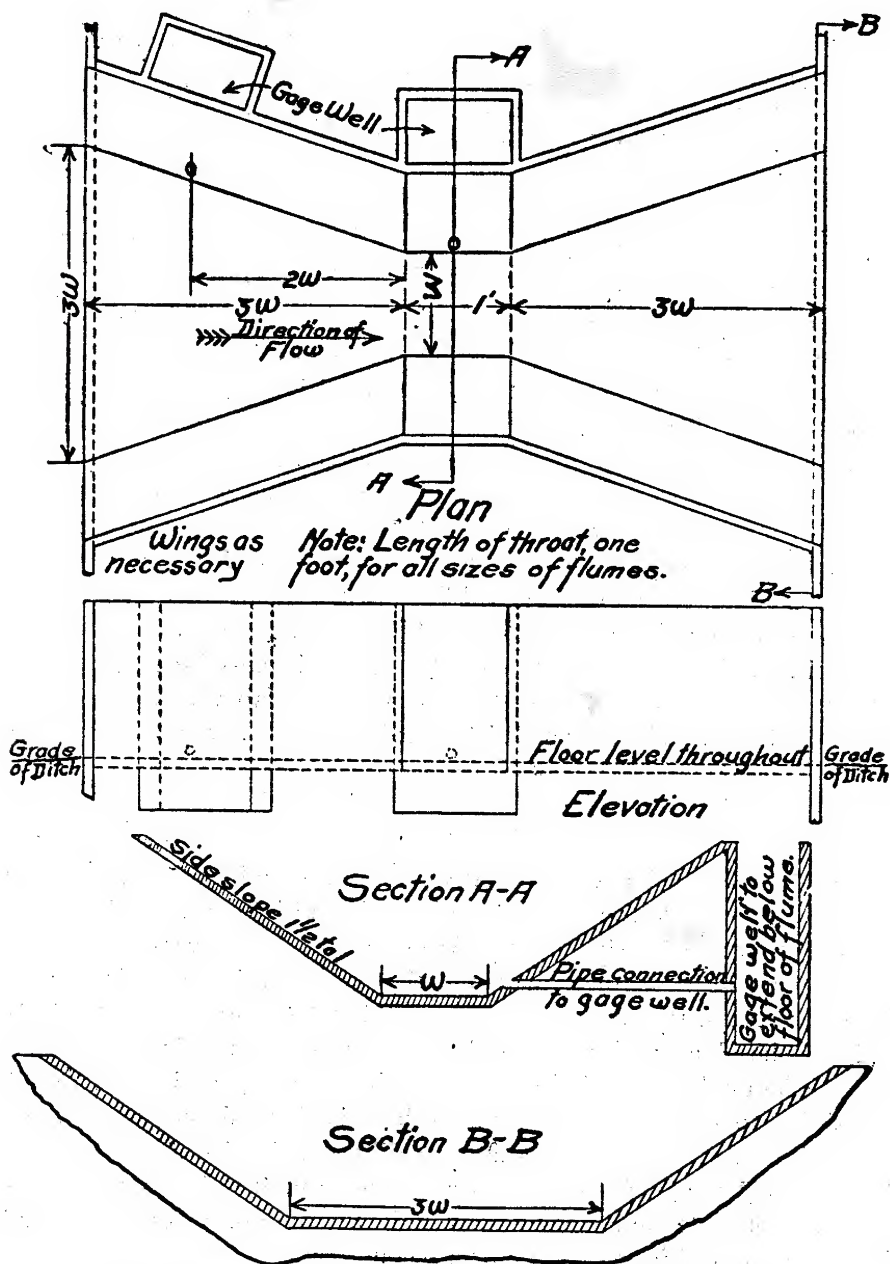


FIG. 6.—Standard plans for the Venturi flume with trapezoidal cross section.



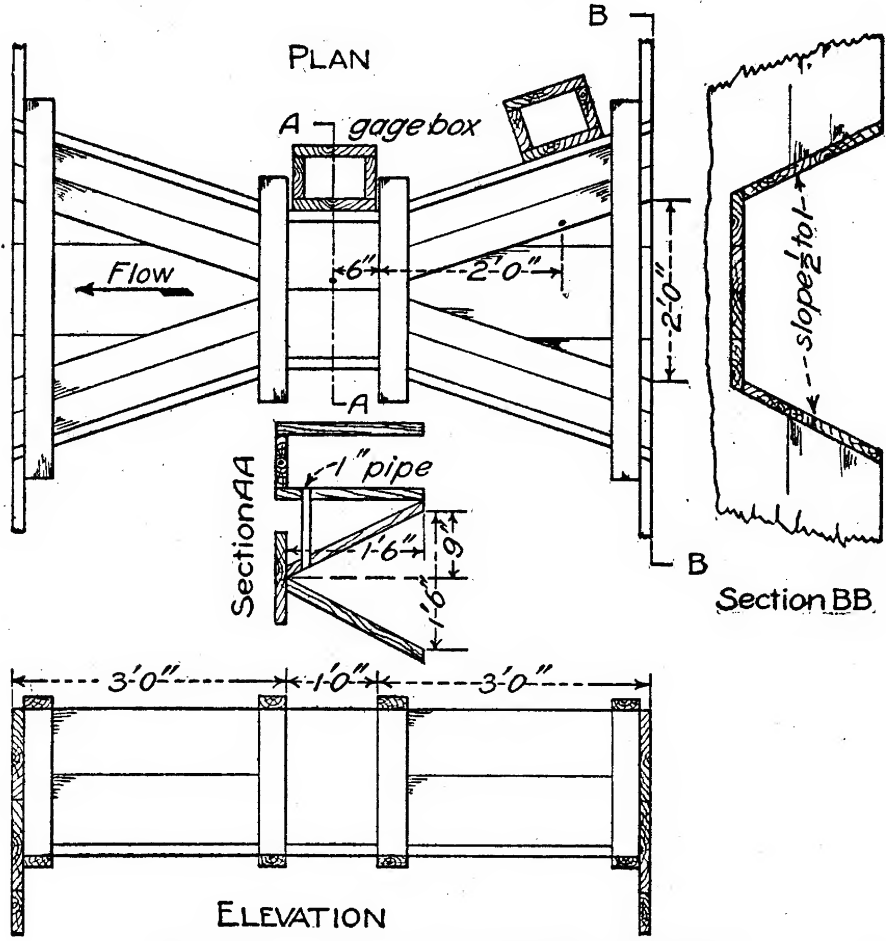


FIG. 7.—Plan, elevation, and sections of the V-notch Venturi flume.

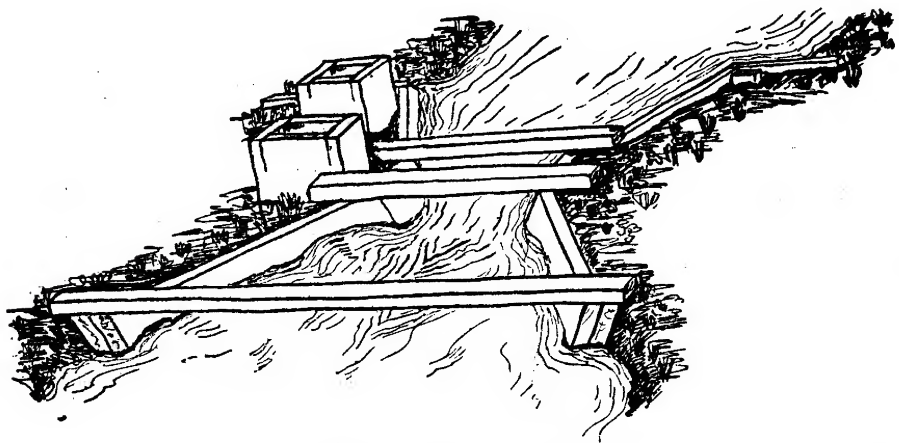


FIG. 8.—Sketch of the Venturi flume, showing installation in ditch.

The effect upon the discharge caused by different arrangements of the channels of approach and recession is shown by the following experimental results. The discharges for each condition have been compared with those for the standard arrangement.

Extending the converging section to a length of approximately 6 feet instead of 3 feet, as in the standard plan (fig. 7), but with the same angle of convergence, caused a decrease in discharge of not to exceed 0.5 per cent for any depth of water.

A channel of approach with parallel sides, having a side slope of  $\frac{1}{2}$  to 1 and a bottom width of 2 feet, joined to the upstream end of the Venturi flume caused a decrease in discharge of less than 1 per cent for any depth of water. This change was comparable to eliminating the  $90^\circ$  wings at the upstream end and joining the device directly to the lined section of a ditch.

With the standard construction for the upstream portion of the flume, a channel of recession similar to the previously described channel of approach was provided. This change had no appreciable effect upon the discharge for any depth of water.

A piece of 2- by 4-inch timber was placed on edge at the upstream end of the flume and nailed to the floor. Its position was normal to the axis of the flume, and it extended across the full width of the section. The increase in discharge due to this change did not exceed 1 per cent for any depth of water.

#### DERIVATION OF FORMULA FOR DISCHARGE THROUGH THE V-NOTCH VENTURI FLUME

From Bernoulli's theorem:

$$\frac{V_a^2}{2g} + p + H_a = \frac{V_b^2}{2g} + p + H_b \quad (1)$$

in which  $V_a$  and  $H_a$  represent the velocity and head at the gage in the upstream section and  $V_b$  and  $H_b$  represent the velocity and head in the throat section.

$$\text{from (1)} \quad V_b^2 = V_a^2 + 2gH_d \quad (2)$$

where  $H_d = H_a - H_b$

$$Q = A_a V_a = A_b V_b$$

$$V_a = \frac{A_b V_b}{A_a} = \frac{\frac{H_b^2 V_b}{2}}{(2\frac{2}{3} + H_a) \frac{H_a}{2}} \quad (3)$$

substituting (3) in (2)

$$V_b^2 = \frac{H_b^4 V_b^2}{(2\frac{2}{3} + H_a)^2 H_a^2} + 2gH_d$$

$$V_b = \sqrt{\frac{2gH_d}{1 - \frac{H_b^4}{(2\frac{2}{3} + H_a)^2 H_a^2}}}$$

and

$$Q = V_b A_b = \frac{H_b^2}{2} \sqrt{\frac{2gH_d}{1 - \frac{H_b^4}{(2\frac{2}{3} + H_a)^2 H_a^2}}} \quad (4)$$

As shown in Table III, discharge values computed by equation (4) are higher than those obtained by experiment, because the equation does not contain a correction factor for the effect of contraction and friction. Table III and figure 9, plotted from this table, show the correction factor ( $C$ ) to be greater for high and low values of  $H_a$  than for medium values of  $H_a$ , and ( $C$ ) increases as  $H_a$  increases. To avoid confusion, coefficients ( $C$ ) for  $H_a$ 's of 1.6 feet, 1 foot, and 0.4 foot, only, have been plotted in figure 9. The assumed limiting curves are shown in dotted

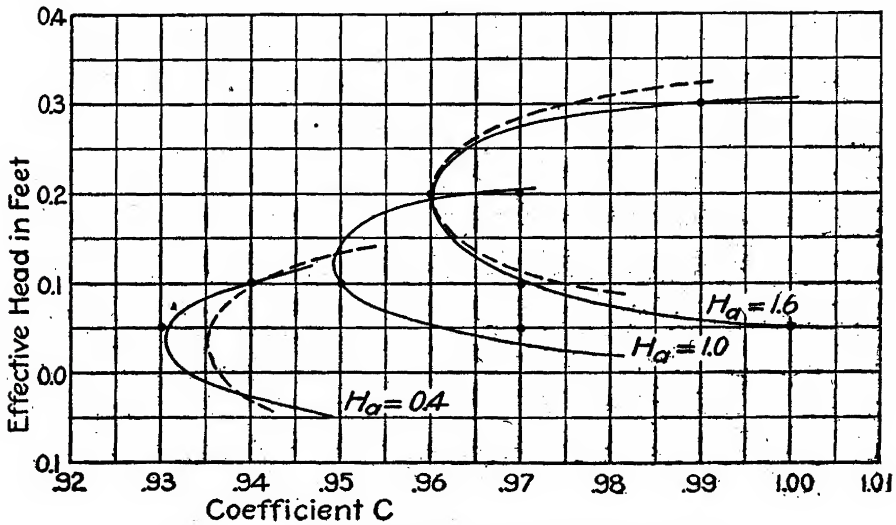


FIG. 9.—Plot of values of coefficient  $C$  for V-notch Venturi flume.

lines. The curves for the intermediate heads were assumed to have a straight line variation between the extreme curves and to change only in position.

The equation of the upper limiting curve,  $H_a = 1.6$  feet, referred to the point 0.96, 0.2 as the origin, is of the form

$$y^n = ax$$

substituting values to find  $n$  and  $a$ ,

$$0.1^n = 0.0167a$$

$$0.03^n = 0.0015a$$

$$\frac{0.1^n}{0.03^n} = \frac{0.0167a}{0.0015a}$$

$$3.33^n = 11.13$$

$$n \log 3.33 = \log 11.13$$

$$n = \frac{\log 11.13}{\log 3.33}$$

$$n = 2.00$$

$$(0.1)^2 = 0.0167a$$

$$a = \frac{0.01}{0.0167} = 0.6$$

The equation is therefore  $y^2 = 0.6x$ . With the origin moved to the point  $o, o$ , the equation of the upper limiting parabola becomes

$$(y - 0.2)^2 = 0.6(x - 0.96) \quad (5)$$

and similarly the equation for the lower limiting curve,  $H_a$ , approxi-

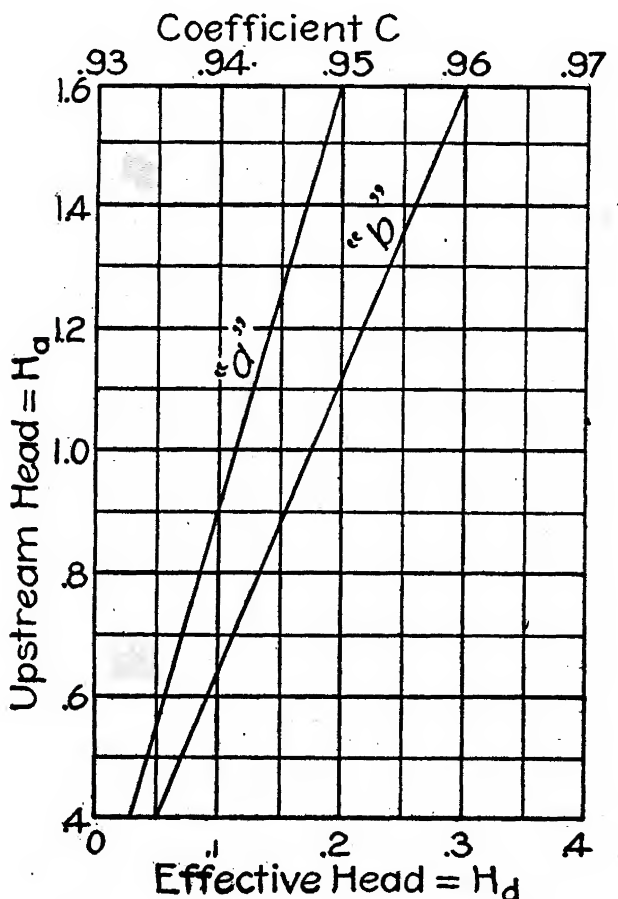


FIG. 10.—Plot of constants for curves shown in figure 9.

mately 0.4 foot, is found to be

$$(y - 0.03)^2 = 0.6(x - 0.935) \quad (6)$$

The constants for each curve were assumed to take a straight line variation, as shown in figure 10, in which the  $a$  line is for the constants with  $y$ , and the  $b$  line is for the constants with  $x$ .

By proportion from figure 10 and the substitution  $H_a$  for  $y$ ,

$$a = 0.14y - 0.02$$

or

$$a = 0.14H_a - 0.02$$

and

$$b = 0.02H_a + 0.93$$

Substituting these values in equation (5) or (6) gives

$$(y - 0.14H_a + 0.02)^2 = 0.6(x - 0.02H_a - 0.93)$$

but since  $y = H_a$  and  $x = c$ ,

$$(H_a - 0.14H_a + 0.02)^2 = 0.6(c - 0.02H_a - 0.93)$$

whence

$$C = \frac{(H_a - 0.14H_a + 0.02)^2 + 0.01H_a + 0.56}{0.6}$$

After the combination of the value of the coefficient  $C$  with the theoretical formula, equation (4) becomes

$$Q = \left[ \frac{(H_a - 0.14H_a + 0.02)^2 + 0.01H_a + 0.56}{0.6} \right] \frac{H_b^2}{2} \sqrt{\frac{29 H_a}{1 - \frac{H_b^4}{(2\frac{2}{3} + H_a)^2 H_a^2}}} \quad (7)$$

in which the bracketed portion represents the coefficient for contraction and friction,  $\frac{H_b^2}{2}$  represents the wetted cross section of the throat of the flume, and the radical expression represents the velocity of flow.

Simplifying the above equation gives

$$Q = 6.68H_b^2[(H_a - 0.14H_a + 0.02)^2 + 0.01H_a + 0.56] \sqrt{\frac{H_a}{1 - \frac{H_b^4}{(2\frac{2}{3} + H_a)^2 H_a^2}}} \quad (8)$$

which is the discharge formula for the V-notch Venturi flume. Table II has been computed for this equation. The experimental discharge values are shown in curve form in figure 5.

Discharge values computed from equation (8), for any given  $H_a$ , increase as  $H_d$  is increased up to a certain point; but with further increase of  $H_d$  the discharge values decrease. At first thought this seems to be impossible; but it must be true, because in the limiting case where  $H_d = H_a$ ,  $H_b$  becomes zero, and from the formula,  $Q$  must equal zero. Discharge values computed from equation (8) must plot into smoothly continuous curves of a reversed character, and these values must therefore ultimately decrease. From equation (7) it is evident that the wetted cross-sectional area of the throat varies as the square of the head,  $H_b$ , while the velocity varies nearly as the square root of the difference in head,  $H_a$ , and therefore for any given  $H_a$ , as the  $H_d$  increases the area decreases more rapidly than the velocity increases.

The calibration experiments with the V-notch Venturi flume did not show any decrease in discharge, such as mentioned above. For each  $H_a$  there is a definite limit to the value of the  $H_d$  which may be obtained



in the practical operation of this device, and it is probable that this limit is about at the reversing point on the discharge curves made from the formula. It was found by experiment that for any given  $H_a$  after a certain  $H_b$  had been obtained, a further lowering of the water surface in the diverging section had no influence upon the elevation of the water at the throat gage,  $H_b$ .

TABLE III.—Comparison of theoretical and experimental discharge values of V-notch Venturi flume

$H_a$	$H_b$	$H_d$	$Q$		$C$
			Experimental.	Computed. <sup>a</sup>	
0.4.....	0.35	0.05	0.102	0.110	0.93
.4.....	.30	.10	.107	.114	.94
1.0.....	.95	.05	.805	.832	.97
1.0.....	.90	.10	.999	1.052	.95
1.0.....	.80	.20	1.124	1.165	.97
1.6.....	1.55	.05	2.300	2.302	1.00
1.6.....	1.50	.10	2.925	3.023	.97
1.6.....	1.40	.20	3.525	3.669	.96
1.6.....	1.30	.30	3.802	3.830	.99

<sup>a</sup> Discharges computed by equation (4), p. 122.

#### TRAPEZOIDAL VENTURI FLUME WITH SIDE SLOPES OF 1 TO 1

This is a special type which was developed to meet a condition common in some sections of the irrigated West, where a ditch is used to carry a small head of water for orchard irrigation at one time and a flow of approximately 10 second-feet for alfalfa irrigation at another time. This requires a quite flexible measuring device, and therefore called for the design shown in figure 11. The side slopes for this type of Venturi flume are 1 to 1, and it is expected that it will be built only in the one size; that is, with a 6-inch bottom throat width. The discharges through this Venturi flume are given in graphic form in figure 12.

The discharge through the Venturi flume with trapezoidal cross section, having side slopes of 1 to 1 in a plane normal to the axis of the flume and with a bottom throat width of 6 inches, is represented by the following equation, which was derived in a manner similar to that given for the V-notch Venturi flume:

$$Q = \left[ \frac{[(H_a - 0.09 H_a - 0.005)^2 + 0.001 H_a + 0.274]}{0.30} \right] \left( \frac{1}{2} + H_b \right) H_b \sqrt{1 - \frac{2g H_a}{\left( \frac{1}{2} + H_b \right)^2 H_b^2} \left( \frac{11}{6} + H_a \right) H_a^2}$$

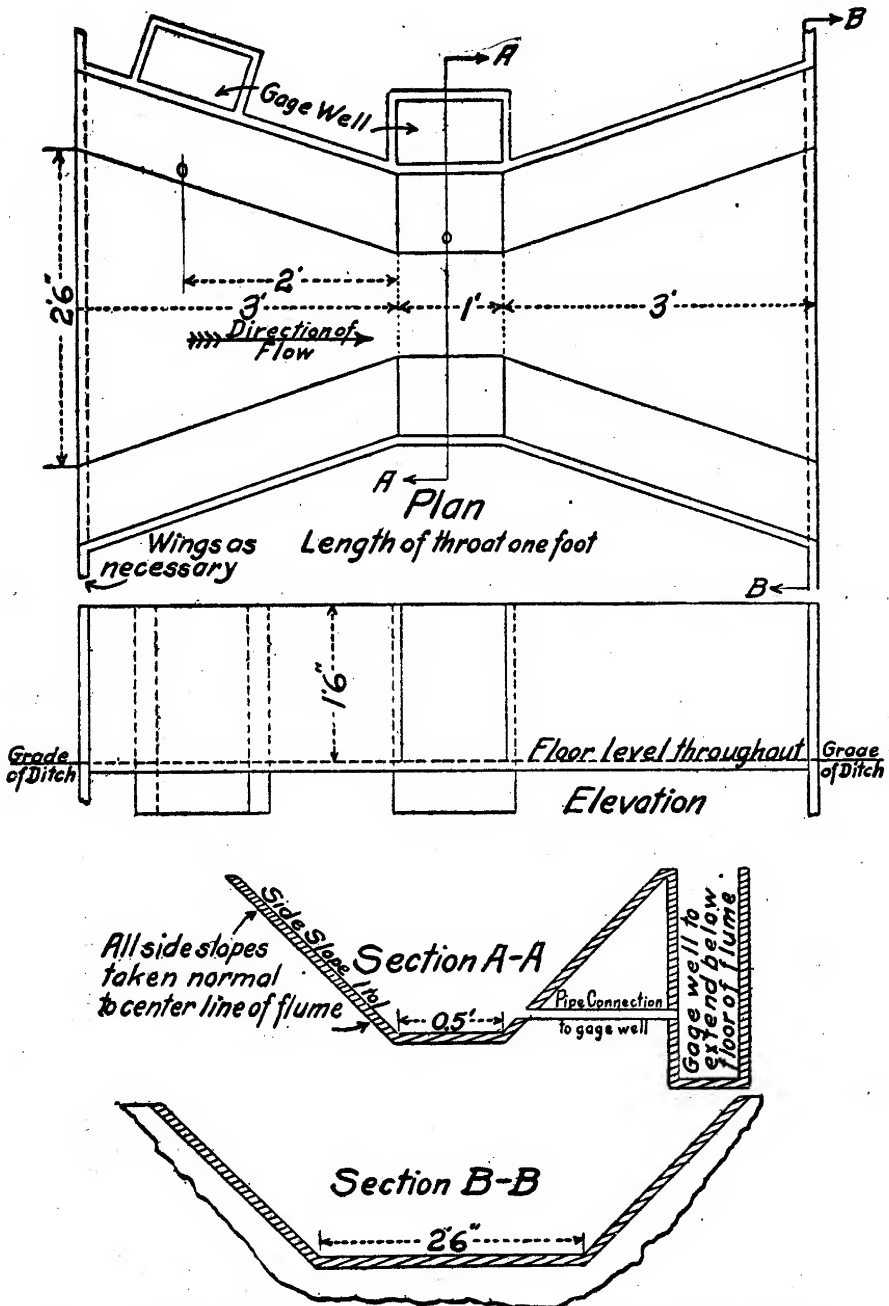


FIG. 11.—Plan for the trapezoidal Venturi flume with 0.5 foot bottom width, side slopes 1 to 1.

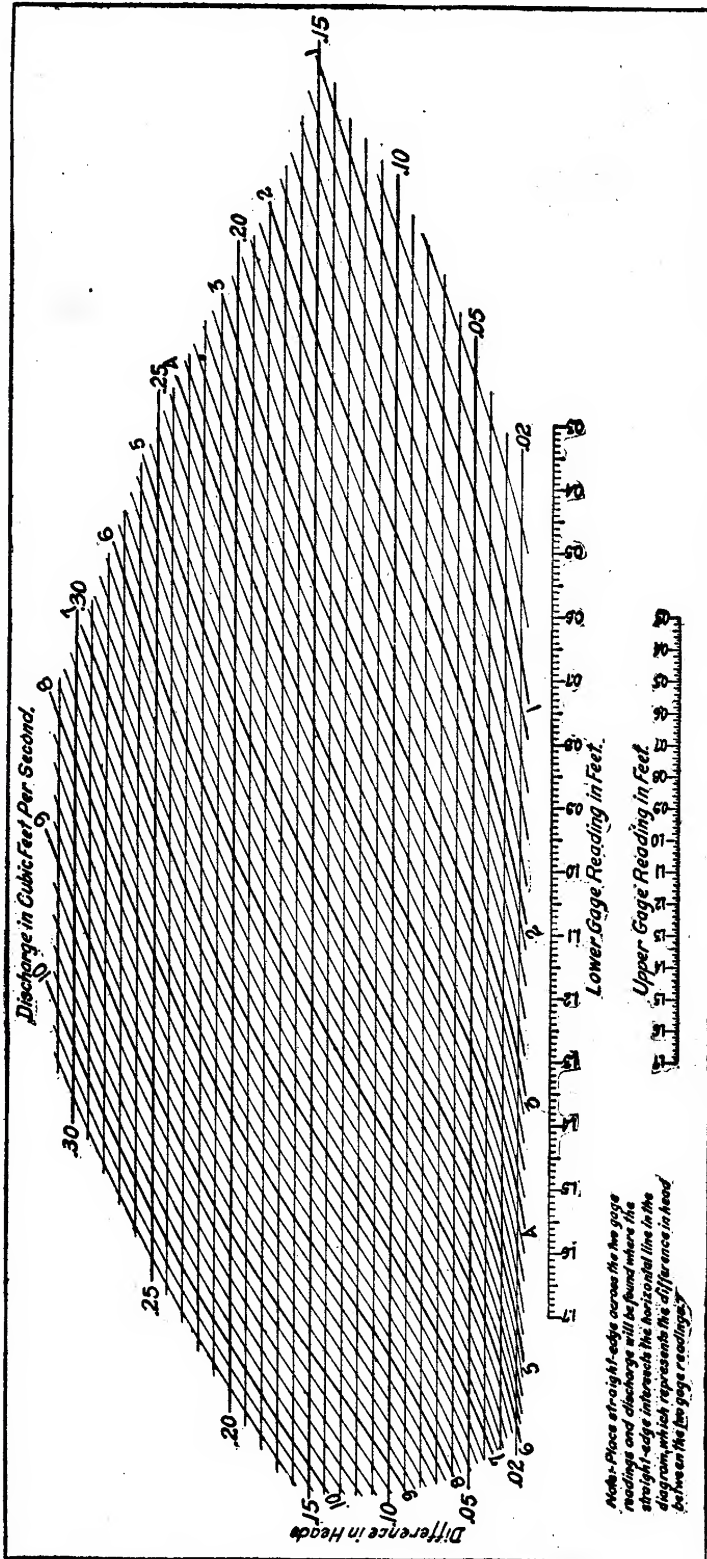


FIG. 12.—Discharge curves for the trapezoidal Venturi flume having 0.5 foot bottom width and side slopes 1 to 1.

## CONCLUSION

The Venturi flume is not an exact measuring device, but it is thought to be sufficiently accurate to meet usual practical needs, especially such as are encountered in irrigation practice in the West.

Although experiments have been made only on the smaller sizes of Venturi flumes, it seems reasonable to expect that structures built according to the general plans will be applicable to the measurement of streams of considerable size, with an accuracy compatible with field requirements.

The Venturi flume seems to fulfill the conditions of being free of trouble from sand, silt, or floating trash; requires little loss of head for making the measurement; is a structure that is simple to build, easy to operate, and has a comparatively low cost; and is free from error in measurement due to aquatic growth or other changes in the channel, provided the floor of the flume is not below the grade of the channel.

If the accompanying discharge curves, formulas, or tables are to be used, it is essential that the Venturi flume be built according to the general plans and the gages for measuring the head be placed as shown in the plans. Alterations of the plans or position of gages will necessitate a recalibration for the new arrangement.

A public patent has been applied for which will permit the manufacture or use of this flume by the public without the payment of royalties.

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## MORPHOLOGY OF NORMAL PIGS' BLOOD<sup>1</sup>

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### INTRODUCTION

The work reported in this paper was undertaken with the view of establishing normal data under conditions which exist in this section of the country as a basis for future studies and to corroborate the work of other investigators.

### HISTORICAL REVIEW

Burnett<sup>2</sup> has compiled a table showing the number of erythrocytes, leucocytes, percentage of hemoglobin, specific gravity, and size of the red corpuscles in the blood of normal swine.

TABLE I.—*Summary of examinations of the blood of normal swine by different investigators (in Burnett<sup>2</sup>)*

Red corpuscles per c. mm.	Leucocytes per c. mm.	Hemoglo- bin.	Specific gravity.	Size of red corpuscles.	Author.
		<i>Per cent.</i>		$\mu$	
6,960,000	7,840	.....	.....	5.28-7.9	Bethe.
7,924,000	19,000	88	.....	6	Giltner.
.....	.....	.....	.....	.....	Gulliver.
5,441,000	.....	.....	.....	.....	Stöltzing.
8,045,000	.....	.....	.....	.....	Storch.
<sup>a</sup> 4,923,000	11,518	.....	.....	.....	Do.
.....	.....	.....	1.060	6	Sussdorf.
8,668,200	.....	.....	.....	.....	Wendelstadt and Bleibtreu.

<sup>a</sup> Pigs 6-35 days old.

The number of examinations upon which the above averages are based is not known, except in the case of Giltner.<sup>3</sup> His original article is available, and his table shows that he made observations upon 24 pigs. From his description the pigs were of fair quality, and were of mixed breeding. Regarding size, they were quite mixed: Five were just weaned, one was a large animal, and the remainder varied in size.

Lewis, Shuler, and others,<sup>4</sup> in connection with their studies on hog cholera, have studied the blood of a few normal susceptible pigs. They

<sup>1</sup> Published, with the approval of the Director, as Paper No. 59 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> Burnett, S. H. *The Clinical Pathology of the Blood of Domesticated Animals.* p. 48. Ithaca, N. Y., 1908.

<sup>3</sup> Giltner, Ward. *The histology and physiology of normal pigs' blood.* *In Jour. Compar. Path. and Ther.*, v. 20, pt. 1, p. 18-23. 1907.

<sup>4</sup> Lewis, L. L., Shuler, W. P., McElroy, C. H., and Ritter, L. B. *Hog cholera.* *Okla. Agr. Exp. Sta. Bul.* 104, 30 p., 9 fig. 1914.

find an average of 8,267,000 erythrocytes per cubic millimeter, 24,500 leucocytes, and 85 per cent of hemoglobin.

Dinwiddie,<sup>1</sup> in his bulletin dealing with a study of the blood of normal and cholera-infected pigs, reports upon 16 normal animals. These pigs were between the ages of 3 and 5 months. He found the average number of red corpuscles to be 6,334,000 and the leucocytes 11,800.

Differential counts of the leucocytes are reported by Drake,<sup>2</sup> Giltner,<sup>3</sup> and Dinwiddie.<sup>4</sup>

Drake, in his studies on trichinosis, made differential counts on 15 normal pigs. He recognizes only three classes of leucocytes. His results are as follows:

Lymphocytes.....	33 to 77 per cent; average, 56.4 per cent.
Polynuclears.....	18 to 66 per cent; average, 38.4 per cent.
Eosinophiles.....	1 to 12 per cent; average, 5.13 per cent.

Giltner recognizes five classes of leucocytes and gives the following percentages based upon 24 examinations:

Lymphocytes.....	30.0 to 79.8 per cent; average, 51.6 per cent.
Large mononuclears.....	.8 to 10.0 per cent; average, 4.6 per cent.
Polymorphs.....	13.0 to 60.0 per cent; average, 37.0 per cent.
Eosinophiles.....	1.2 to 11.0 per cent; average, 5.2 per cent.
Mast cells.....	.2 to 5.6 per cent; average, 1.3 per cent.

Dinwiddie gives the following percentages based upon 16 examinations:

Mononuclears.....	24 to 81 per cent; average, 59.0 per cent.
Polymorphs.....	16 to 75 per cent; average, 35.0 per cent.
Transitional.....	1 to 6 per cent; average, 2.9 per cent.
Eosinophiles.....	1 to 10 per cent; average, 4.0 per cent.
Basophiles.....	1 to 2 per cent; average, 1.1 per cent.

#### METHODS OF STUDY

The 25 young pigs used in this work were obtained from three litters of pigs born from healthy sows and were farrowed on the university farm. They were healthy, strong pigs of mixed breeding. The pigs ranged in age from 2 to 42 days, and in weight from 2.5 to 18 pounds. The age, weight, sex, and condition are included in Table II.

The larger pigs were selected from animals which had been purchased upon the open market at the South St. Paul stock yards, and were to be used for serum production. The pigs came from various points throughout the Northwest, and when a load was received a number of the pigs were selected for this work. The pigs selected were of good quality. They were first weighed and tagged, then given a few days' rest in a small inside pen. During this time they received feed and water three

<sup>1</sup> Dinwiddie, R. R. Studies on the hematology of normal and cholera-infected hogs. *Ark. Agr. Exp. Sta. Bul.* 120, p. 21-41, 8 fig. 1914.

<sup>2</sup> Drake, A. K. Trichinosis. *In Jour. Med. Research*, v. 8 (n. s. v. 3), no. 1, p. 255-267, 1 pl. 1902.

<sup>3</sup> Giltner, Ward. *Op. cit.*

<sup>4</sup> Dinwiddie, R. R. *Op. cit.*

times daily. If the pigs showed any sickness, as evidenced by loss of appetite or increase in body temperature, they were not used. All of the pigs listed in Table III were afterwards used in producing hog-cholera virus.

The young pigs were taken from the pen, in which they were confined with their mother, to the operating room, and the various tests made or samples collected; following this, the young pigs were returned to the mother. The samples of blood were taken from the small veins in the ear, after applying alcohol and then shaving. The puncture was made with a sharp scalpel.

The samples from the larger pigs were obtained in a similar manner, except that these pigs were confined in a special hog crate. The samples were obtained as nearly as possible at the same hour each day, 10 a. m.

The puncture produced a good flow of cutaneous blood, and the first few drops were wiped away. It was usually necessary to make several punctures before completing the examination.

The usual methods of study were employed. The percentage of hemoglobin was obtained by means of the Sahli hemometer. The blood for estimating the number of erythrocytes and leucocytes was drawn in the same pipette, and Toisson's diluting fluid used as the diluting mixture. Two pipettes giving a dilution of 1 to 100 were used in each case, and both erythrocytes and leucocytes were counted on the same field. A count was made from each pipette, and, if the amount of variation was 100,000 erythrocytes or less, the results were considered satisfactory and were recorded. The counts were made on the Thoma-Zeiss hematocytometer counting chamber with the Zappert-Ewing ruling. The leucocytes were sometimes estimated by the acetic-acid method, with a pipette of 1 to 10 dilution. The specific gravity was determined by the Hammerschlag method. The time of coagulation was determined by means of the Biffi-Brooks coagulometer.

In making the differential counts of the leucocytes Wright's stain was employed as the staining agent, and 200 to 300 of the corpuscles counted on at least two spreads of blood, so that never less than 500 leucocytes were counted.

#### RESULTS OF EXPERIMENTATION

Twenty-five examinations were made of the blood of normal pigs between the ages of 2 and 42 days. The results of these examinations are shown in Table II. The average number of erythrocytes was 3,855,000 per cubic millimeter, and 13,500 leucocytes per cubic millimeter. The average clotting time was 64 seconds, specific gravity 1.024, and the average hemoglobin percentage 56.8. Differential count of leucocytes showed the following: Lymphocytes, 63.25 per cent; polymorphs, 32.14 per cent; mononuclears, 2.63 per cent; eosinophiles, 1.28 per cent; mast cells, 0.24 per cent.

TABLE II.—Details of blood studies on 25 young pigs

Description of animal.				Blood.					Differential count.					
No.	Age.	Sex.	Weight.	Condi- tion.	Clotting time.	Hemoglobin.	Specific gravity.	Number of ery- throcytes.	Number of leu- cocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Mast cells.
	Days.		Lbs.		Secs.	P. ct.				P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1.....	2	Male.....	2.5	Good.....	55			2,864,000	8,000	48.38	47.44	3.03	0.94	0.18
2.....	5	do.....	6.0	Very good.	70			3,598,000	15,000	63.46	31.45	4.52	.56	.0
3.....	5	do.....	5.0	Good.....	50			2,560,000	22,000	51.18	44.29	4.13	.39	.39
4 <sup>a</sup> .....	5	do.....	do.....	do.....	62			2,240,000	22,000	49.00	43.50	5.22	1.08	.09
5 <sup>a</sup> .....	5	do.....	do.....	do.....	70			2,240,000	22,000	79.50	18.00	2.70	1.0	.8
6.....	7	Female.....	6.0	do.....	50			3,404,000	10,000	72.40	25.17	1.97	.01	.0
7 <sup>a</sup> .....	7	do.....	do.....	do.....	37			2,308,000	5,000	63.50	30.50	3.00	.42	1.6
8.....	8	do.....	5.0	do.....	60			3,068,000	11,000	58.95	38.21	2.18	.65	.0
9.....	11	do.....	6.0	do.....	60			4,300,000	13,000	58.76	40.07	1.15	.0	.0
10.....	15	Male.....	7.0	do.....	60			3,315,000	.....	75.73	22.81	1.27	.18	.0
11.....	19	do.....	8.0	do.....	80			1.035 3,378,000	10,000	84.70	14.57	0.36	.36	.01
12.....	22	do.....	8.0	do.....	38			1.013 2,528,000	11,000	85.02	13.67	0.02	.37	.0
13 <sup>a</sup> .....	28	do.....	12.0	do.....	65			4,376,000	10,000	76.00	20.40	1.80	1.10	.33
14 <sup>a</sup> .....	28	Female.....	15.0	do.....	60			4,688,000	18,000	77.00	15.00	3.90	2.70	.0
15 <sup>a</sup> .....	28	Male.....	18.0	do.....	90			5,220,000	20,000	80.20	6.30	2.80	8.50	.1
16.....	28	do.....	8.0	do.....	47			4,970,000	.....	60.00	37.30	2.70	.0	.0
17.....	32	do.....	10.0	do.....	60			5,994,000	6,000	51.00	44.00	0.025	.018	.002
18.....	33	Female.....	10.0	do.....	40			2,420,000	11,000	58.13	33.70	3.50	.46	.0
19.....	33	Male.....	10.0	do.....	50			4,784,000	10,000	66.42	27.28	2.87	1.07	.52
20 <sup>a</sup> .....	35	Female.....	do.....	do.....	46			2,680,000	10,000	56.00	40.00	2.20	1.40	.70
21.....	35	do.....	10.0	do.....	60			4,602,000	13,000	61.40	34.88	1.86	1.40	.46
22.....	37	do.....	14.0	Very good.	60			5,032,000	12,000	52.93	43.15	2.93	.81	.16
23.....	40	do.....	13.0	Good.....	90			4,622,000	15,000	60.60	35.40	3.00	1.00	.0
24.....	40	Male.....	10.0	do.....	65			4,380,000	20,000	33.79	63.39	3.63	.17	.0
25.....	42	do.....	10.0	do.....	100			5,136,000	26,000	57.70	33.93	4.09	3.44	.82
Average.....					64.0	56.8	1.024	3,855,000	13,500	63.25	32.1365	2.63	1.2872	.246

<sup>a</sup> Examinations made by Dr. L. E. Willey.

Twenty-five examinations were made upon the blood of pigs weighing in the neighborhood of 100 pounds. The results of this examination are shown in Table III. The average number of erythrocytes per cubic millimeter was 6,215,000, and the average number of leucocytes per cubic millimeter was 18,000. The average clotting time was 57.60 seconds, specific gravity 1.062, and the average hemoglobin percentage was 79.4. The differential count of leucocytes showed the following: Lymphocytes, 55.21 per cent; polymorphs, 39.79 per cent; mononuclears, 0.79 per cent; eosinophiles, 3.42 per cent; mast cells, 0.79 per cent.

TABLE III.—Details of blood studies on 25 older pigs

Description of animal.					Blood.					Differential count.				
No.	Weight.	Sex and color.	Condi- tion.	Temperature.	Clotting time.	Hemoglobin.	Specific gravity.	Number of ery- throcytes.	Number of leu- cocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Mast cells.
	Lbs.				Sec.	P. ct.				P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
6618	77	White and black fe- male.	Good.....		45	70	1.046	6,536,000	15,000	62.15	34.72	0.86	2.08	0.17
6627	99	Red and black fe- male.	...do.....		45	55	1.064	5,798,000	14,000	48.78	47.96	1.46	1.62	.16
6692	88	White fe- male.	...do.....		70	48	1.046	5,656,000	24,000	47.89	50.42	.16	.84	.67
6714	70	...do.....	...do.....		50	75	1.027	6,344,000	17,000	69.85	28.49	.18	.55	.90
6772	92	...do.....	...do.....		50	74	1.053	6,704,000	17,000	66.90	28.93	.36	3.43	.47
6765	97	Red female.	...do.....		60	72	1.048	6,012,000	15,000	59.68	37.20	1.21	1.90	.00
5992	80	Brown and black male	...do.....		60	105	1.060	7,268,000	15,000	55.88	42.40	123	2.47	.00
6032	76	...do.....	...do.....		60	90	1.065	7,080,000	16,000	33.50	64.39	1.32	.75	.00
6224	88	Black female	...do.....		100	72	1.050	5,658,000	20,000	38.81	56.37	1.09	2.21	1.47
6225	86	...do.....	...do.....		90	80	1.063	6,872,000	19,000	51.68	38.94	2.83	6.19	.35
6981	74	Black and white fe- male.	...do.....	102.8	65	70	1.070	5,640,000	14,000	50.65	46.75	1.30	.52	.78
7073	101	White male.	...do.....	99.3	70	70	1.063	5,156,000	33,000	42.40	51.42	.16	5.52	.47
7015	101	Female.	...do.....	102.4	70	78	1.070	6,831,800	14,000	60.90	25.73	.00	10.00	3.34
7071	108	Red female.	...do.....	102.4	70	65	1.069	6,196,000	17,000	50.97	46.09	.42	2.37	.14
7160	85	White male.	...do.....	101.6	55	70	1.064	5,272,000	19,000	42.25	55.36	1.21	.00	.17
7162	88	White fe- male.	...do.....	103.2	40	70	1.070	5,456,000	19,000	53.09	43.43	.58	2.31	.58
7216	111	Black male.	...do.....	103.6	70	70	1.066	5,520,000	18,000	56.92	35.86	.19	5.88	1.14
7217	102	Black female	...do.....	102.8	35	70	1.071	6,380,000	15,000	62.83	28.32	.36	5.13	3.37
7273	105	Black male.	...do.....	102.4	55	70	1.067	5,960,000	15,000	70.62	24.48	.19	4.33	.37
7271	105	Black female	...do.....	103.6	35	70	1.070	6,104,200	18,000	68.43	25.42	.34	4.26	1.53
7272	130	...do.....	...do.....	103.5	20	100	1.066	5,624,000	28,000	55.98	38.01	.38	4.82	.19
6308	110	White female	...do.....	102.2	60	115	1.071	7,568,000	17,000	58.88	40.18	.37	.52	.00
7276	110	...do.....	...do.....	102.8	75	112	1.069	6,217,000	20,000	.....	.....	.....	.....	.....
7278	110	Black and white male	...do.....	101.8	60	102	1.066	5,976,000	20,000	57.05	26.61	1.40	13.11	2.09
7279	120	Red female.	...do.....	103.3	30	112	1.070	7,580,000	19,000	59.17	37.04	1.54	1.37	.85
Average.....					57.60	79.40	1.06176	6,215,160	18,320	52.21	39.79	.79	3.42	.79

In making the differential counts we have used a classification of the leucocytes similar to the one proposed by Giltner.<sup>1</sup> Drake<sup>2</sup> recognizes only three varieties of leucocytes, but we agree with Giltner in that five varieties can be easily distinguished in a well-made and well-stained spread. Dinwiddie<sup>3</sup> also recognizes five classes. He classifies all the mononuclear elements under one head, and gives the transitional forms a special classification.

The following is a somewhat detailed description of the various classes observed in our studies.

LYMPHOCYTES.—Burnett<sup>4</sup> states that the cells falling in this group have practically the same appearance in the majority of domesticated animals. With Wright's stain the cell body has a greenish blue tint, while the nucleus has a dark-violet tint. Giltner<sup>1</sup> describes two kinds

<sup>1</sup> Giltner, Ward. Op. cit.

<sup>2</sup> Drake, A. K. Op. cit.

<sup>3</sup> Dinwiddie, R. R. Op. cit.

<sup>4</sup> Burnett, S. H. Op. cit., p. 35.



of lymphocytes in the blood of normal pigs, the first variety slightly larger than the red cells, generally spherical in shape, from 7.4 to 10  $\mu$  in diameter, and averaging about 8.5  $\mu$ . They have a nucleus which occupies nearly the whole cell, showing in most cases a crescent-shaped darker or lighter blue portion of the cell body at the periphery. The second variety includes much larger cells, 11 to 14  $\mu$  in diameter. They are fewer in number, have round nuclei, and occupy relatively less of the cell space.

Giltner's classification, therefore, is the same as the classification of large and small lymphocytes often referred to in the literature when discussing this particular class of cells in other animals.

In our studies we have been able to recognize three classes of lymphocytes. The cells of the first class are few in number and about one-half the size of a red cell. The nucleus is round, deeply stained, and occupies almost the entire cell space. These cells were observed in the blood of both young and older animals. The cells of the second class, the most numerous by far, are a little larger. They are two to three times as large as a red cell, the nucleus is stained dark, and a small amount of bluish green cytoplasm can be recognized. The cells of the third class of lymphocytes are still larger; they have a slightly larger nucleus and a larger amount of visible protoplasm. It is sometimes difficult to distinguish between these cells and the smaller of the so-called large mononuclears.

No attempt was made to work out the various percentages of these three classes of lymphocytes; they were, therefore, all classed as lymphocytes. It would be extremely difficult to work out the percentages of these three classes, and the results would be subject to much error, owing to the fact that many border-line cells present themselves, and it would be difficult to know into just what class to place some of them.

**LARGE MONONUCLEARS.**—Burnett states these cells are usually about twice the diameter of the average red corpuscles. The nucleus usually occupies only about one-half of the cell and is situated on one side of the center. Its shape is oval or curved. Both nucleus and cell body are finely reticular and stain less deeply than do those of the lymphocytes. The cell body is faintly basophile. The cells have much the same appearance in the several species of domestic animals.

Giltner<sup>1</sup> states that in the pig the more typical large mononuclear leucocytes are similar in size to the large lymphocytes and have a medium-sized nucleus, light blue and bean-shaped, and show a large portion of the cell body stained a different shade of blue.

The cells which we have classified as large mononuclear leucocytes vary in size from a size equal to, or a little larger than, the largest lymphocytes up to very large cells, the largest of which are 5 times as long and 5

<sup>1</sup> Giltner, Ward. *Op. cit.*

times as wide as an average red corpuscle. In recognizing these cells two things are important: First, the great size of some of the cells, and second, the lighter stained nucleus than the lymphocytes and the larger amount of visible cytoplasm. This cytoplasm in a well-stained spread is light bluish green in color, with a few fine darker granules throughout.

We have found that it is sometimes difficult to determine whether to place some of these mononuclear cells into the class of lymphocytes or large mononuclears, as there are quite a number of border-line cells.

**POLYMORPHONUCLEARS.**—The nucleus in this variety is several-lobed; the lobes are nearly always connected, sometimes with threadlike connections, but more often the connecting portions are as wide as the lobes, so that the nucleus assumes the form of a deeply stained spiral coil. It may be roughly S-shaped or Z-shaped. In well-stained spreads the cell body contains many fine granules so small that they appear as brick-red points. The entire cell is about the size of the largest lymphocytes. Sometimes the lobes of the nucleus are not connected and stand out in such a way that they can be easily counted, and average about six to seven in number.

**EOSINOPHILES.**—Giltner states that the eosinophiles are comparable to the polymorphs in size, or are slightly larger, and have a bilobed nucleus, the two parts of which are connected by a thick band and take the basic stain. The cell body is granular, but the granules are not nearly so large and distinct as those found in the eosinophile of the horse, but are more numerous (estimated at 100, more or less) and have a strong affinity for the eosin stain.

In our work we have found that the eosinophiles are usually slightly larger than the polymorphs. The nucleus is darkly stained, but is lighter than the nucleus of the polymorphs. The lobes are wide, and there may be one to four lobes in each nucleus. The lobes are usually connected by broad bands, but we sometimes find a nucleus containing two large lobes which are not connected. No attempt was made to count the number of granules.

**MAST CELLS.**—Giltner<sup>1</sup> states the mast cells in the blood of the pig are about the same size as the eosinophiles and have a similar-shaped nucleus, but that the cell body possesses granules of a smaller size, more distinct in outline, and of a purple color. The granules lie both in the cytoplasm surrounding the nucleus and in a position superposed to the nucleus.

In the blood of some pigs we have found mast cells about the size of eosinophiles, which closely resemble a large lymphocyte except that the space in the cell body not occupied by the nucleus is filled with dark-blue granules. Granules are also quite numerous over the nucleus in many of these cells, but the number of granules placed in this location

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<sup>1</sup> Giltner, Ward. Op. cit.

varies widely. These granules are not as large as the red granules found in the eosinophiles.

Gruner<sup>1</sup> speaks of this kind of a mast cell as a true mast cell. Such cells have a narrow cytoplasm. The granules vary in size. He states that these cells are lymphocytes which have undergone mucoid degeneration.

In the blood of some pigs the mast cells are quite large, the nucleus usually bilobed, a large amount of cell space is visible, and the granules are slightly larger but not as numerous as in the cells first described.

TRANSITIONALS.—Dinwiddie<sup>2</sup> recognizes a classification of transitional leucocytes. Occasionally true and distinct transitional forms of leucocytes are met with in the blood of the pig, but we have followed the custom of most workers and have classified them in one group of the related type. True transitionals would very likely represent about 1 to 2 per cent of the total leucocytes, but there would be many border-line cases, and it would be difficult to know just where to place them. Individual workers would very likely differ widely in classifying them, and we have found it much easier to place them in the recognized group which they most closely resembled. Therefore, when these cells were encountered, they were either added to the large mononuclears, lymphocytes, or polymorphs.

BLOOD PLATELETS.—No attempt was made to estimate the number of blood platelets per cubic millimeter. Blood plates occurring singly and in clumps are often encountered in the dry spread. When Wright's stain is used, the blood plates take a bluish tint and show many dark granules. The blood platelets which are encountered singly vary in size and shape. They may be round, oval, or irregular; some are smaller, and others are larger than an average-sized red corpuscle. When in clumps, the individual size, shape, etc., can not be determined.

ERYTHROCYTES.—In a well-stained spread the erythrocytes are red in color and are circular. Willey, working in this laboratory, found nucleated red corpuscles in the blood of a 4-weeks-old pig.

EFFECTS OF AGE.—Observations made by different workers relatively to the effects of age upon the number of red blood cells are conflicting. Storch<sup>3</sup> reports the number in adult swine to be 8,045,000 and in young pigs (6 to 35 days old) 4,923,000.

Giltner's<sup>4</sup> table shows a higher red-cell count in young pigs (2.5 months) than in the older animals, averaging 8,363,333 erythrocytes for the young and 7,322,500 for the older. The leucocytic count was about the same in the two groups.

Our results are more in accord with those of Storch. The red corpuscles showed an average of 3,853,071 cells per cubic millimeter in the

<sup>1</sup> Gruner, O. C. *Biology of the Blood-Cells* . . . p. 259. London, 1913.

<sup>2</sup> Dinwiddie, R. R. *Op. cit.*

<sup>3</sup> Burnett, S. H. *Op. cit.*, p. 48. Cites paper by Storch.

<sup>4</sup> Giltner, Ward. *Op. cit.*

25 young pigs and 6,176,272 in the older hogs. The leucocytic count was also lower in the young animals, averaging 12,328 in the young and 18,533 in the older animals.

Burnett<sup>1</sup> states that the lymphocytes in young animals are generally present in greater numbers than in adults. He states that in puppies 3 to 20 days old they were 20.8 to 30.7 per cent, while for adults they average 19.4 per cent. We have found a similar condition in the pig. In young pigs the lymphocytes average 62.25 per cent and in adults 55.21 per cent. The large mononuclears are also present in greater numbers in the young animals, 2.63 per cent being found in young animals and 0.75 per cent in older animals. The polymorphs, eosinophiles, and mast cells are present in greater numbers in adults. These cells show a percentage of the polymorphs of 39.79 in older animals and 32.13 in young animals; of the eosinophiles, 3.42 in the old animals and 1.28 in the young; of the mast cells, 0.79 in old animals and 0.24 in the young.

Giltner<sup>2</sup> found a higher percentage of hemoglobin in the older animals, 88 in the older and 85 in the younger.

Our results also show a higher percentage of hemoglobin in the older animals. The older animals averaged 79 per cent, as compared with 66 per cent in the younger animals.

We also found a higher specific gravity in the case of the older pigs, and the clotting time was about 6 seconds shorter in these older animals.

**DIFFERENCES OF SEX.**—Burnett<sup>3</sup> states that the number of red corpuscles and the amount of hemoglobin seem to be higher in male than female domestic animals.

Giltner's results show about the same number of red corpuscles in the blood for females and males. They average 7,945,000 for the males and 7,895,500 for the females. He found the leucocytes to be a little higher in the male. These cells average 18,150 in the male and 16,415 in the female. Giltner found the percentage of hemoglobin to be about equal in the two sexes, 87 in the male and 88 in the female.

Our results show the number of erythrocytes to be about equal in both groups (young and old). In young pigs the average number of red corpuscles was 3,953,071 in the males, and 3,718,400 in the females. In the older pigs the average number of red corpuscles was 6,068,250 in the males, and 6,284,294 in the females. We found a higher leucocytic count in the males than females. The young males showed an average white-cell count of 12,857, as compared with 11,800 in the females. The older males showed an average white-cell count of 19,125, as compared with 17,941 in the females.

We found a higher percentage of hemoglobin in the male animals of both groups. In the case of the young animals the males showed 60 per cent, as compared with 52.5 per cent in the female. The older males

<sup>1</sup> Burnett, S. H. Op. cit.

<sup>2</sup> Giltner, Ward. Op. cit.

<sup>3</sup> Burnett, S. H. Op. cit., p. 58.

showed an average of 81.5 per cent as compared with 78 per cent in the older females. The average differential counts for the two sexes showed no important differences (Table IV).

TABLE IV.—*Variation in the differential count in male and female pigs*

Age.	Male.					Female.				
	Lym- pho- cytes.	Poly- morphs.	Large mononu- clears.	Eosino- philes.	Mast cells.	Lym- pho- cytes.	Poly- morphs.	Large mono- nu- clears.	Eosino- philes.	Mast cells.
Young..	64. 14	31. 22	2. 67	1. 28	0. 216	61. 96	33. 61	2. 57	1. 30	0. 29
Old.....	53. 81	41. 12	. 73	4. 07	. 64	56. 05	39. 13	. 83	3. 10	. 87

#### SUMMARY

(1) The number of erythrocytes in the blood of the pig varies under different conditions. It is lower in young animals than in old.

(2) The number of erythrocytes also varies according to the condition of the animal. A well-nourished pig in good condition will show a higher count than a pig in poor condition and of the same age.

(3) The number of erythrocytes was about equal in the blood of male and female animals.

(4) The leucocyte count was lower in young animals, but individuals of the same class may show considerable variation.

(5) The number of leucocytes seems to be higher in male than in female animals.

(6) The percentage of hemoglobin was higher in older animals.

(7) The percentage of hemoglobin was higher in male than female animals.

(8) The specific gravity of the blood was higher in older animals.

(9) The clotting time was less in younger animals.

(10) Five classes of leucocytes can be recognized in the blood of the pig: Lymphocytes, large mononuclears, polymorphonuclears, eosinophiles, and mast cells.

(11) Results of the differential counts by various workers are fairly uniform.

(12) The percentage of lymphocytes and large mononuclears is higher in young animals.

(13) Older animals show a higher percentage of polymorphonuclears, eosinophiles, and mast cells than young animals.

(14) Differential counts in male and female animals are about the same.



# FIXATION OF AMMONIA IN SOILS<sup>1</sup>

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## INTRODUCTION

It is well known that the capacity of a soil to serve as a reservoir for plant food depends in a large measure upon its power to retain water-soluble substances, such as potash, phosphoric acid, and ammonia, against the leaching action of rains.

There are undoubtedly many forces which operate in the retention of soluble salts in soils, and it is quite likely that these forces are very different in different soils. The extremely complicated nature of the phenomena has led to many apparently conflicting observations; and it would seem that an entirely satisfactory explanation of the fixation processes can not be secured until more refined methods have been developed which will make possible a more exact knowledge of the physical and chemical forces involved.

In studying the nitrifying power of semiarid soils at different depths, it was observed that, when ammonium sulphate was added to soils drawn from considerable depths, little or no increase in nitrates resulted during the first two weeks' incubation; but, notwithstanding the lack of nitrification, the ammonia added could not be recovered from the soil by any of the ordinary methods for determining ammonia in soils. In the early work only small quantities of ammonium sulphate were added, and it was thought possible that the ammonia might have been assimilated by microorganisms. Larger quantities of ammonium sulphate were then added. Little or no increase in nitrates was secured, but only a small percentage of the ammonia added could be recovered from the soils drawn from a considerable depth. The results obtained with the surface soil were quite different, about 95 per cent of the nitrogen added being recovered as ammonia or nitrates. This observation seemed to indicate a strong ammonia-fixing power for the deep soil layers, a condition which, if true, would lead to complications in studying the ammonifying and nitrifying power of the soils at different depths.

The work reported in this paper is limited to a study of the fixation of ammonia by soils, and is an outgrowth of the observations stated above.

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<sup>1</sup> The work discussed in this paper was carried out in cooperation with the University of California Citrus Experiment Station and Graduate School of Tropical Agriculture at Riverside. The writer wishes to express his indebtedness to Director H. J. Webber and members of his staff for many courtesies and facilities extended during the course of the work.

## METHODS OF EXPERIMENTATION

The soil samples employed in the investigations reported in this paper were secured by means of soil-sampling tubes or, where large quantities of soil were desired, by digging a hole to the desired depth and removing a uniform block of soil from the side. All the soils studied were passed through a coarse sieve, after which they were thoroughly mixed. One-hundred-gm. portions of dry soil were then weighed into 1-quart

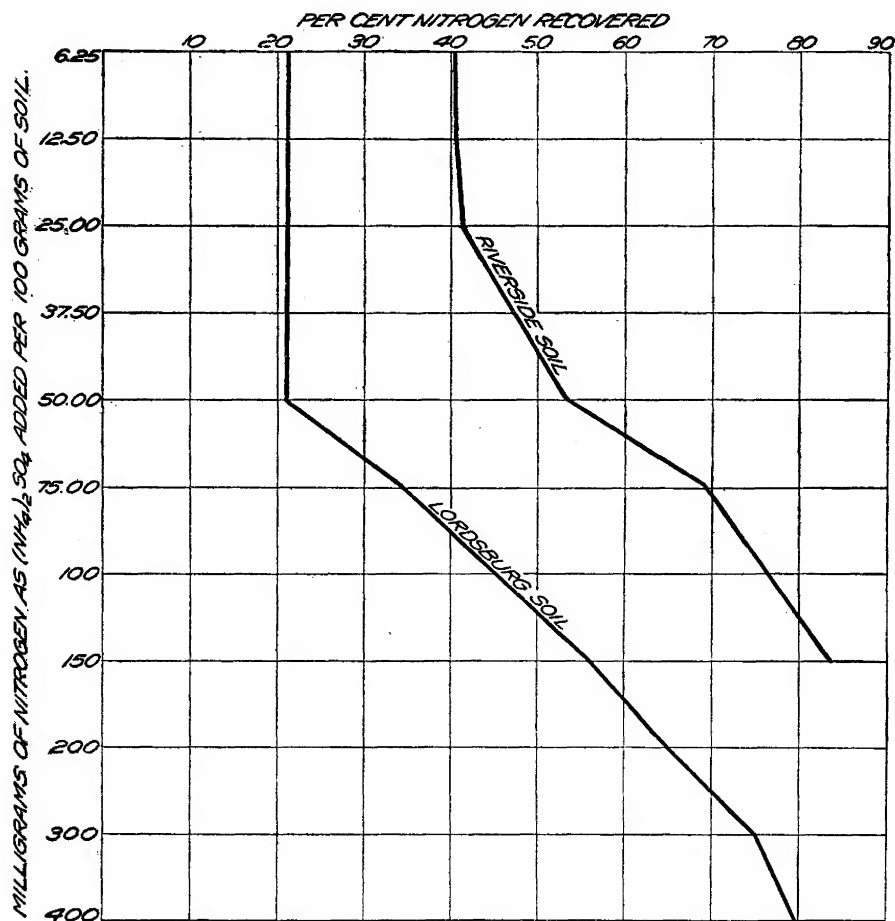


FIG. 1.—Diagram showing the percentage of ammonia recovered from soils when increasing amounts of ammonium sulphate were added.

Mason jars. The desired amount of ammonium salts or other salts was added in standard solutions in such quantities that the solution added was sufficient to saturate the soil thoroughly.

Unless otherwise stated in the text, the ammonium solution was allowed to remain in contact with the soil for 30 minutes. The soil was then extracted by a 10 per cent hydrochloric-acid solution and an aliquot portion of the extract, rendered alkaline by adding sodium-

hydroxid solution, was distilled. The distillate was titrated with alizarin.

The acid-extraction method was used in preference to direct distillation with magnesium oxid, because duplicate determinations by acid extraction gave a much closer agreement than could be secured by distilling the soil with magnesium. Ten per cent of acid was used, as it was found that this amount of acid was necessary to extract as much ammonia as would be given off by distilling with magnesium oxid.

The results stated in the following tables are averages of a number of determinations. In no case is a result for a single analysis given, and in some instances the figures as given are averages of six or more determinations.

#### COMPARISON OF METHODS FOR RECOVERY OF AMMONIA FROM SOILS

Fifty mgm. of nitrogen in the form of ammonium sulphate, chlorid, or nitrate were added to 100-gm. portions of soil from Riverside, Cal. After standing for 30 minutes, the soils were treated as shown in Tables I and II. When the soil was extracted with water only, but little more than one-fourth of the nitrogen added was recovered. When extracted with increasing strengths of hydrochloric acid, the percentage of nitrogen recovered increased with the strength of the acid; but even when the acid solution was increased to 10 per cent, only a little more than half of the nitrogen added was recovered.

TABLE I.—*Recovery of ammonia from soil by extracting with water and increasing the amount of hydrochloric acid*

Soil extracted with—	Nitrogen added per 100 gm. of soil.	Nitrogen recovered from ammonium sulphate.		Nitrogen recovered from ammonium chlorid.		Nitrogen recovered from ammonium nitrate.	
		Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
Water only.....	50.00	12.88	25.76	13.28	26.56	13.00	26.00
1 per cent acid.....	50.00	22.12	44.24	22.33	44.66	22.30	44.60
2½ per cent acid.....	50.00	22.68	45.36	23.30	46.60	23.20	46.40
5 per cent acid.....	50.00	24.36	48.72	24.74	49.48	24.50	49.00
10 per cent acid.....	50.00	26.04	52.08	25.76	51.52	25.76	51.52

The acid-extraction method and the magnesium-oxid method for determining ammonia in soils are compared in Table II. A glance, first, at the Riverside soil will show that between 52 and 53 per cent of the nitrogen added was recovered by either method. In the Lordsburg soil the percentage of nitrogen recovered is much smaller than in the Riverside soil, and it would seem that the magnesium-oxid method was slightly more effective than the acid-extraction method, although the difference is small. It is observed that the ammonia fixed is practically the same whether the ammonia is added as a sulphate, chlorid, or nitrate. It would therefore appear that the anions have no effect on the fixation of ammonia.

TABLE II.—Comparison of direct-distillation and acid-extraction methods for determining ammonia in soils

Ammonium salts added.	Nitro- gen added per 100 gm. of soil.	Nitrogen recovered.							
		Riverside soil.				Lordsburg soil.			
		Extracted with 10 per cent of hydrochloric acid.		Extracted with 10 gm. of mag- nesium oxid.		Extracted with 10 per cent of hydrochloric acid.		Extracted with 10 gm. of mag- nesium oxid.	
		Mgm.	Per ct.	Mgm.	Per ct.	Mgm.	Per ct.	Mgm.	Per ct.
Ammonium hydroxid. ....	50.00	26.16	52.32	26.20	52.40	10.10	20.20	11.00	22.00
Ammonium nitrate. ....	50.00	26.28	52.56	26.30	52.60	10.00	20.00	10.80	21.60
Ammonium chlorid. ....	50.00	26.16	52.32	26.40	52.80	10.10	20.20	10.75	21.50
Ammonium sulphate. ....	50.00	26.30	52.60	26.50	53.00	8.50	17.00	9.10	18.20

After it became evident that the ammonia could not be removed from the soil by extracting with acid or distilling with magnesium oxid, the soil was distilled with increasing amounts of potassium and sodium hydroxid. The results secured are presented in Table III. It is seen that the ammonia removed by boiling with potassium and sodium hydroxid is greater than the amount recovered by extracting with acid or distilling with magnesium oxid; but that much of the ammonia was retained by the soil, even when boiled with large quantities of potassium and sodium hydroxid. One c. c. of 10*N* potassium-hydroxid solution seemed to be less effective than 2.5 c. c., but the maximum removal of ammonia seems to have been secured when 2.5 c. c. of solution were added. In some cases the sodium hydroxid seems to have been more effective than the potassium solution; but this is possibly due to the fact that duplicate samples were quite variable, and, even though the results given are averages of several determinations, some allowance must be made for the lack of uniformity in duplicates. It is obvious that much of the ammonia fixed by this soil can not be removed by boiling with caustic substances, even when the compounds are added in great excess. When the soils were boiled with 10 per cent hydrochloric acid, more of the ammonia was removed than by extracting with cold hydrochloric acid of the same strength (Table IV). However, boiling for one hour left about one-third of the ammonia in the Riverside soil. When the boiling was continued for four hours, the ammonia removed varied from 86.4 to 88 per cent. The ammonia seems to have been more firmly fixed in the Lordsburg soil, as boiling for four hours with 10 per cent acid removed less than 75 per cent of the nitrogen added.

TABLE III.—*Recovery of ammonia from soils by distilling with potassium hydroxid and sodium hydroxid*

Quantity of potassium-hydroxid or sodium-hydroxid solution added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
		10N potassium hydroxid.		10N sodium hydroxid.	
C. c.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.
1.....	50.00	31.92	63.84	33.60	67.20
2.....	50.00	34.72	69.44	36.96	73.92
5.....	50.00	34.66	69.32	36.12	72.24
10.....	50.00	34.72	69.44	36.96	73.92
20.....	50.00	34.72	69.44	36.68	73.36
30.....	50.00	34.44	68.88	37.24	74.48
40.....	50.00	34.68	69.36	36.72	73.44
80.....	50.00	34.44	68.88	34.16	68.32
100.....	50.00	34.48	68.96	34.60	69.20

TABLE IV.—*Recovery of ammonia from soil by boiling with 10 per cent hydrochloric acid*

Time of boiling.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered.											
		Riverside soil.						Lordsburg soil.					
		Ammonium sulphate.		Ammonium chlorid.		Ammonium nitrate.		Ammonium sulphate.		Ammonium chlorid.		Ammonium nitrate.	
Hours.	Mgm.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.
1.....	50.00	32.40	64.80	33.00	66.00	32.60	65.20						
2.....	50.00	37.60	75.20	37.40	74.80	38.00	76.00	25.50	51.00	26.00	52.00	25.60	51.20
3.....	50.00	40.24	80.48	40.00	80.00	40.80	81.60						
4.....	50.00	43.20	86.40	43.75	87.50	44.00	88.00	36.00	72.00	36.40	72.80	37.00	74.00

## NITROGEN RECOVERED

The amount of ammonia recovered from soils by leaching with water or 10 per cent hydrochloric acid is shown in Table V.

TABLE V.—*Removal of ammonia from soils by leaching with water or 10 per cent hydrochloric acid*

Quantity of percolate.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Riverside soil.				Lordsburg soil.	
		Leached with water.		Leached with 10 per cent hydrochloric acid.		Leached with water.	
C. c.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
400.....	50.00	11.42	22.84	25.80	51.60	4.40	8.80
800.....	50.00	14.63	29.26	34.04	68.08	5.30	10.60
1,200.....	50.00	15.54	31.08	37.68	75.36	5.90	11.80
1,600.....	50.00	16.15	32.30	39.06	78.12	6.50	13.00
2,000.....	50.00	16.39	32.78	40.06	80.12	6.90	13.80
2,400.....	50.00	16.50	33.00	40.60	81.20	7.10	14.20



The percolation of 400 c. c. of water through 100 gm. of soil removed 22.84 per cent of the ammonia from the Riverside soil and only 8.8 per cent from the Lordsburg soil. The percolation of the 400 c. c. of 10 per cent acid through the Riverside soil removed a little more than twice as much ammonia as the same amount of water. The leaching of the soils was continued until 2,400 c. c. of percolate had passed through. The total amount of ammonia removed by leaching with water amounted to only 14.2 per cent for the Lordsburg soil and 33 per cent for the Riverside soil.

The removal of ammonia by leaching with large quantities of 10 per cent acid was tried only with the Riverside soil, from which 2,400 c. c. removed 81.2 per cent of the ammonia added.

#### FACTORS INFLUENCING THE FIXATION OF AMMONIA BY SOILS

As shown in Table VI, the depth from which the soil sample is taken is frequently an important factor in the determination of the ammonia-fixing power of the soil. All of the semiarid soils tested, with the exception of the soil from Highland, Cal., show an increased fixation with an increase in depth. The humid soils do not show an increased fixation with depth, and it would therefore seem that there may be a marked difference between humid and semiarid soil in this regard. However, the number of soils examined are too few to warrant any conclusions at this time.

TABLE VI.—*Fixation of ammonia by soils from different depths*

Depth of soil.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.													
		Lordsburg, Cal.		Riverside, Cal.		Covina, Cal.		Highland, Cal.		Berwyn, Md.		Arlington, Va.			
												Sample 1.		Sample 2.	
Inches.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
0-6...	50.00	47.20	94.40	46.50	93.00	44.40	88.80	49.50	99.00	48.20	96.40	45.40	90.80	45.40	90.80
6-18...	50.00	38.90	77.80	45.85	91.70	42.15	84.30	46.95	93.90	48.70	97.40	45.00	90.00	46.00	92.00
18-30...	50.00	21.80	43.60	40.80	81.60	36.05	72.10	47.30	94.60	47.60	95.20	46.40	92.80	46.40	92.80
30-42...	50.00	12.90	25.80	33.85	67.70	22.35	44.70	.....	.....	47.20	94.40	.....	.....	.....	.....
42-54...	50.00	10.10	20.20	26.70	53.40	.....	.....	.....	.....	46.00	92.00	.....	.....	.....	.....

The addition of the same quantity of an ammonium salt in concentrated and dilute solution shows the greatest fixation when added in concentrated solution, as shown in Table VII. When dilute solutions were added, the containers were placed on a large wheel which completed about two revolutions per minute; thus, the soil was kept agitated dur-

ing the fixation period. However, it is observed that the nitrogen recovered varied from 52 to 53.5 per cent when 20 c. c. of solution were added and from 65 to 66 per cent when 720 c. c. of solution were added.

TABLE VII.—*Fixation of ammonia from concentrated and dilute solutions*

Quantity of solution added.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered from ammonium sulphate.		Nitrogen recovered from ammonium chlorid.		Nitrogen recovered from ammonium nitrate.	
C. c.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
20.....	50.00	26.10	52.20	26.25	53.50	26.00	52.00
200.....	50.00	27.52	55.04	28.10	56.20	27.80	55.60
360.....	50.00	28.25	56.50	29.00	58.00	28.80	57.60
720.....	50.00	32.61	65.22	32.60	65.20	33.00	66.00

If the fixation is dependent upon the chemical reactions, it would seem that the temperature would be an important factor in determining the amount of ammonia fixed. Table VIII shows the results secured with two soils when held at temperatures ranging from 5° to 100° C. In both soils the amount of ammonia recovered decreased as the temperature increased. In the Lordsburg soil the ammonia recovered when the soil was held at 100° is only 53.6 per cent of the amount recovered when the soil was held at 5°.

TABLE VIII.—*Effect of temperature on the fixation of ammonia by soils*

Temperature.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
		Riverside soil.		Lordsburg soil.	
°C.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.
5.....	50.00	27.42	55.44	12.50	25.00
20.....	50.00	25.48	50.96	10.10	20.20
50.....	50.00	20.16	40.32	9.10	18.20
75.....	50.00	19.32	38.64	8.80	17.60
100.....	50.00	17.36	34.72	6.70	13.40

The time of standing exerted some influence, as can be seen by referring to Table IX. As might be expected, the most rapid fixation takes place during the first few minutes after the ammonia is added; but the process is apparently not complete even after a period of 72 hours, as less ammonia was removed after 96 hours than after 72 hours; and in the Lordsburg soil the difference is quite appreciable.

TABLE IX.—*Effect of time on the fixation of ammonia by soils*

Time ammonia was allowed to remain in contact with the soil.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
		Riverside soil.		Lordsburg soil.	
Hours.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.
½	50.00	20.82	41.64	10.10	20.20
1	50.00	26.50	53.00	9.90	19.80
4	50.00	25.46	50.92	9.10	18.20
8	50.00	24.02	48.04	7.90	15.80
16	50.00	23.00	46.00	7.70	15.40
24	50.00	22.57	45.14	7.75	15.50
48	50.00	21.84	43.68	7.80	15.60
72	50.00	21.26	42.52	7.60	15.20
96	50.00	20.97	41.94	6.60	13.20

Heating a soil to high temperatures caused marked changes in the chemical and physical nature of the soil, as shown in Table X. A temperature of 200° C. or less for six hours seems to have had very little effect, but 250° caused a marked reduction in the ammonia-fixing power of the soil, and a temperature of 300° reduced the fixation in the Riverside soil to 10 per cent and in the Lordsburg soil to 12.8 per cent.

TABLE X.—*Effect of heating the soil in a hot-air oven for six hours previous to the addition of ammonia*

Temperature.	Nitrogen added per 100 gm. of soil.	Nitrogen recovered.					
		Ammonium sulphate.				Ammonium chlorid.	
		Riverside soil.		Lordsburg soil.		Riverside soil.	
°C.	Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
100	50.00	25.76	51.52	10.40	20.80	25.76	51.52
150	50.00	25.76	51.52	10.35	20.70	25.76	51.52
200	50.00	26.88	53.76	10.60	21.20	26.60	53.20
250	50.00	37.24	74.48	33.10	66.20	37.19	74.38
275	50.00	42.92	85.84	39.20	78.40	42.64	85.28
300	50.00	45.00	90.00	43.60	87.20	45.00	90.00

An examination of Table XI shows that the percentage of nitrogen as ammonia recovered remained constant until the amount of nitrogen added was greater than 25 mgm. per 100 gm. of Riverside soil. After this point the percentage fixation decreased, but the absolute fixation increased until about 23 mgm. per 100 gm. of soil were fixed, after which no further increase was secured. The percentage fixation in the Lordsburg soil remained constant until the nitrogen added amounted to more than 50 mgm. per 100 gm. of soil. When amounts of nitrogen greater

than 50 mgm. per 100 gm. of soil were added, the percentage fixation decreased as the amount added was increased. It would seem that the Riverside soil has the power of fixing about 900 pounds of nitrogen as ammonia per acre-foot, while the Lordsburg soil is capable of fixing more than 3,000 pounds. These figures are based upon the fixation which took place in 30 minutes. If the ammonia had been allowed to remain in contact with the soil for several days, the amount fixed would have been very greatly increased, as shown in Table IX.

TABLE XI.—Effect of adding increasing amounts of ammonium sulphate on the fixation of ammonia by soils

Nitrogen in ammonium sulphate added per 100 gm. of soil.	Riverside soil.			Lordsburg soil.		
	Nitrogen recovered.	Nitrogen fixed.	Nitrogen recovered.	Nitrogen recovered.	Nitrogen fixed.	Nitrogen recovered.
Mgm.	Mgm.	Mgm.	Per cent.	Mgm.	Mgm.	Per cent.
6.25.....	2.52	3.73	40.32	2.80	9.70	22.40
12.50.....	5.04	7.46	40.32	5.60	19.40	22.40
25.00.....	10.36	14.64	41.44	8.40	29.10	22.40
37.50.....	17.80	19.70	47.48	11.20	38.80	22.40
50.00.....	26.60	23.40	53.20	25.95	49.05	34.60
75.00.....	52.00	23.00	69.33	44.80	55.20	44.80
100.00.....	76.44	23.56	76.44	84.00	66.00	56.00
150.00.....	125.50	24.50	83.67	129.60	70.40	64.80
200.00.....				224.40	75.60	74.80
300.00.....				318.00	82.00	79.50
400.00.....						

EFFECT OF ALUMINIUM, IRON, POTASSIUM, SODIUM, MAGNESIUM, AND CALCIUM SALTS ON THE FIXATION OF AMMONIA BY SOILS

The addition of aluminium salts to the soil 30 minutes prior to the addition of the ammonium salt caused a marked reduction in the ammonia-fixing power of the soil. Even the addition of 10 c. c. of *N*/<sub>32</sub> aluminium sulphate, chlorid, or nitrate reduced the ammonia-fixing power of the soil, as shown in Table XII. When 10 c. c. of *N*/<sub>1</sub> aluminium salts were added, about 90 per cent of the ammonia added was recovered. The addition of the aluminium salt 24 hours prior to the addition of the ammonium salts generally caused a greater reduction in fixation than when the aluminium salt was allowed to act but one-half hour. However, the time factor is of much less importance than the amount of salt added. The soluble iron and potassium salts, as shown in Tables XIII and XIV, also have a marked influence on the ammonia-fixing power of the soil tested. The potassium salts appear to have had somewhat less effect than the aluminium or iron salts when added in very small quantities; but, when added in larger quantities, these salts were quite as effective as the aluminium or iron salts.

TABLE XII.—Effect of aluminium salts on the fixation of ammonia in a soil from Riverside, Cal.

Time aluminium salt was allowed to stand before addition of ammonia.	Quantity and strength of aluminium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Aluminium sulphate.		Aluminium chlorid.		Aluminium nitrate.	
Hours.		Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
1/2.....	10 c. c. N/1..	50.00	45.15	90.30	43.96	87.92	44.20	88.40
1/2.....	10 c. c. N/2..	50.00	42.35	84.70	41.44	82.88	42.00	84.00
1/2.....	10 c. c. N/4..	50.00	40.67	81.34	40.60	81.20	40.70	81.40
1/2.....	10 c. c. N/8..	50.00	37.87	75.74	37.80	75.60	37.60	75.20
1/2.....	10 c. c. N/16..	50.00	31.41	62.82	33.88	67.76	31.80	63.60
1/2.....	10 c. c. N/32..	50.00	28.63	57.26	29.96	59.92	29.00	58.00
24.....	10 c. c. N/1..	50.00	47.00	94.00	46.70	93.40	46.80	93.60
24.....	10 c. c. N/2..	50.00	44.52	89.04	44.60	89.20	44.80	89.60
24.....	10 c. c. N/4..	50.00	42.56	85.12	42.70	85.40	42.60	85.20
24.....	10 c. c. N/8..	50.00	38.92	77.84	38.90	77.80	39.00	78.00
24.....	10 c. c. N/16..	50.00	33.60	67.20	33.40	66.80	33.60	67.20
24.....	10 c. c. N/32..	50.00	28.84	57.68	28.60	57.20	28.70	57.40

TABLE XIII.—Effect of adding iron salts on the fixation of ammonia in a soil from Riverside, Cal.

Time iron salt was allowed to stand before addition of ammonia.	Quantity and strength of iron salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.			
			Iron chlorid.		Iron nitrate.	
Hours.		Mgm.	Mgm.	Per cent.	Mgm.	Per cent.
1/2.....	10 c. c. N/1..	50.00	43.96	87.92	45.71	91.42
1/2.....	10 c. c. N/2..	50.00	42.28	84.56	43.75	87.50
1/2.....	10 c. c. N/4..	50.00	41.16	82.32	40.39	80.78
1/2.....	10 c. c. N/8..	50.00	36.12	72.24	34.23	68.46
1/2.....	10 c. c. N/16..	50.00	32.20	64.40	24.33	48.66
1/2.....	10 c. c. N/32..	50.00	29.40	58.80	27.80	55.60
24.....	10 c. c. N/1..	50.00	47.32	94.64	47.00	94.00
24.....	10 c. c. N/2..	50.00	46.20	92.40	46.30	92.60
24.....	10 c. c. N/4..	50.00	41.32	82.64	40.90	81.80
24.....	10 c. c. N/8..	50.00	34.44	68.88	34.80	69.60
24.....	10 c. c. N/16..	50.00	31.20	62.40	31.40	62.80
24.....	10 c. c. N/32..	50.00	28.70	57.40	28.60	57.20



TABLE XIV.—*Effect of adding potassium salts on the fixation of ammonia in soils from Riverside, Cal.*

Time potassium salt was allowed to act before the addition of ammonia.	Quantity and strength of potassium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Potassium sulphate.		Potassium chlorid.		Potassium nitrate.	
Hours.		Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
1/2.....	10 c. c. N/1..	50.00	47.09	94.18	47.48	94.96	47.09	94.18
1/2.....	10 c. c. N/2..	50.00	42.56	85.12	42.56	85.12	42.74	85.48
1/2.....	10 c. c. N/4..	50.00	34.72	69.44	34.72	69.44	34.72	69.44
1/2.....	10 c. c. N/8..	50.00	30.80	61.60	30.80	61.60	30.80	61.60
1/2.....	10 c. c. N/16.	50.00	28.00	56.00	28.00	56.00	28.00	56.00
1/2.....	10 c. c. N/32.	50.00	26.60	53.20	26.60	53.20	26.60	53.20
24.....	10 c. c. N/1..	50.00	47.32	94.64	47.25	94.50	47.40	94.80
24.....	10 c. c. N/2..	50.00	42.56	85.12	43.00	86.00	42.60	85.20
24.....	10 c. c. N/4..	50.00	38.64	77.28	38.60	77.20	38.50	77.00
24.....	10 c. c. N/8..	50.00	32.48	64.96	32.50	65.00	32.60	65.20
24.....	10 c. c. N/16.	50.00	29.12	58.24	29.10	58.20	29.10	58.20
24.....	10 c. c. N/32.	50.00	27.72	55.44	27.60	55.20	27.80	55.60

The action of sodium salts on the fixation of ammonia is quite different from the action of potassium salts, as can be readily seen by comparing Tables XIV and XV. The addition of sodium salts seems to have slightly reduced the ammonia-fixing power of the soil. When the sodium salts were added 24 hours prior to the addition of the ammonia, the reduction in the fixing power of the soil was no greater than when the sodium salt was allowed to act only one-half hour. The addition of magnesium and calcium salts, like the sodium salts, caused very little change in the ammonia-fixing power of the soil, even when the salts were added in considerable amounts (Tables XVI and XVII).

It appears that the anions have little or no effect in determining the action of a salt on the ammonia-fixing power of a soil. The three cations which had a marked influence on the ammonia-fixing power of a soil were equally effective, regardless of the anion with which they were combined; and the action of the three cations which had little effect was apparently not influenced by the anions with which they were combined.

TABLE XV.—Effect of adding sodium salts on the fixation of ammonia by a soil from Riverside, Cal.

Time sodium salt was allowed to stand before the addition of ammonia.	Quantity and strength of sodium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Sodium sulphate.		Sodium chlorid.		Sodium nitrate.	
Hours.		Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
1/2.....	10 c. c. N/1..	50.00	27.24	54.48	27.16	54.32	27.46	54.92
1/2.....	10 c. c. N/2..	50.00	27.10	54.20	27.20	54.40	27.26	54.52
1/2.....	10 c. c. N/4..	50.00	27.10	54.20	27.16	54.32	27.16	54.32
1/2.....	10 c. c. N/8..	50.00	27.30	54.60	27.24	54.48	27.06	54.12
1/2.....	10 c. c. N/16..	50.00	27.15	54.30	27.36	54.72	27.20	54.40
1/2.....	10 c. c. N/32..	50.00	27.20	54.40	27.16	54.32	27.16	54.32
24.....	10 c. c. N/1..	50.00	26.80	53.60	26.76	53.52	26.10	52.20
24.....	10 c. c. N/2..	50.00	26.00	52.00	26.76	53.52	26.00	52.00
24.....	10 c. c. N/4..	50.00	26.75	53.50	26.76	53.52	26.80	53.60
24.....	10 c. c. N/8..	50.00	26.90	53.80	26.48	52.96	26.90	53.80
24.....	10 c. c. N/16..	50.00	26.00	52.00	26.34	52.68	26.75	53.50
24.....	10 c. c. N/32..	50.00	26.85	53.70	26.20	52.40	26.90	53.80

TABLE XVI.—Effect of adding magnesium salts on the fixation of ammonia in a soil from Riverside, Cal.

Time magnesium salt was allowed to stand before the addition of ammonia.	Quantity and strength of magnesium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Magnesium sulphate.		Magnesium chlorid.		Magnesium nitrate.	
Hour.		Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
1/2.....	10 c. c. N/2..	50.00	26.88	53.76	26.74	53.48	26.88	53.76
1/2.....	10 c. c. N/4..	50.00	26.70	53.40	26.70	53.40	27.00	54.00
1/2.....	10 c. c. N/8..	50.00	26.60	53.20	26.60	53.20	26.70	53.40
1/2.....	10 c. c. N/16..	50.00	26.40	52.80	26.80	53.60	26.60	53.20
1/2.....	10 c. c. N/32..	50.00	26.32	52.64	26.60	53.20	26.80	53.60

TABLE XVII.—Effect of adding calcium salts on the fixation of ammonia by a soil from Riverside, Cal.

Time calcium salt was allowed to stand before the addition of ammonia.	Quantity and strength of calcium salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.					
			Calcium sulphate.		Calcium chlorid.		Calcium nitrate.	
Hour.		Mgm.	Mgm.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.
1/2.....	10 c. c. N/1..	50.00	26.70	53.40	26.60	53.20	26.80	53.60
1/2.....	10 c. c. N/2..	50.00	26.80	53.60	26.32	52.64	27.16	54.32
1/2.....	10 c. c. N/4..	50.00	26.80	53.60	26.40	53.80	26.70	53.50
1/2.....	10 c. c. N/8..	50.00	26.50	53.00	26.70	53.40	26.60	53.20
1/2.....	10 c. c. N/16..	50.00	26.40	52.80	26.50	53.00	26.40	52.80
1/2.....	10 c. c. N/32..	50.00	26.60	53.20	26.40	52.80	26.30	52.60

Table XVIII shows that the three salts which had little influence on the fixation of ammonia in the soil from Riverside, Cal., also had little effect on the ammonia-fixing power of the soil from Lordsburg, Cal., while the iron, potassium, and aluminium salts, which had a marked influence on the ammonia-fixing power of the Riverside soil, were also effective in the Lordsburg soil. It would therefore seem that the process of fixation in these two soils is somewhat closely related. However, the relative effectiveness of the iron, potassium, and aluminium salts appears to be somewhat different in the two soils.

TABLE XVIII.—Effect of nitrate salts on the fixation of ammonia by a Lordsburg soil

Time nitrate salts were allowed to stand before the addition of ammonia.	Quantity and strength of nitrate salt added.	Nitrogen in ammonium sulphate added per 100 gm. of soil.	Nitrogen recovered.													
			Calcium nitrate.		Magnesium nitrate.		Sodium nitrate.		Ferric nitrate.		Potassium nitrate.		Alumi- nium nitrate.			
Hour.		Mgm.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.	Mgm.	P. ct.
1/2	5 c. c. N/2..	50.00	11.60	23.20	11.50	23.00	11.40	22.80	33.90	67.80	22.00	44.00	38.00	76.00		
1/2	10 c. c. N/2.	50.00	11.80	23.60	11.70	23.40	11.40	22.80	42.00	84.00	33.50	67.00	41.60	83.20		

EFFECT OF SALTS ON THE SOLUBILITY OF CALCIUM IN SOILS

During the course of the work on the fixation of ammonia by soils it was observed that large quantities of calcium were brought into solution, and it was thought that the calcium dissolved by the addition of ammonium salts might bear some relation to the ammonia fixed by the soil. Table XIX shows the effect of ammonium, aluminium, sodium, and magnesium chlorids on the solubility of calcium in semiarid and humid soils. It is seen that the solutions of these salts invariably dissolved a much larger quantity of calcium than did distilled water.

TABLE XIX.—Effect of ammonium, aluminium, sodium, and magnesium chlorid on the solubility of calcium in semiarid and humid soils

[Results stated as milligrams of calcium per 100 gm. of soil]

Locality of sample.	Depth from which sample was taken.	Quantity of each salt solution added.	Water only.	Increase over water only.						
				NH <sub>4</sub> Cl.	AlCl <sub>3</sub> .	AlCl <sub>3</sub> + NH <sub>4</sub> Cl.	NaCl.	NaCl + NH <sub>4</sub> Cl.	MgCl <sub>2</sub> .	MgCl <sub>2</sub> + NH <sub>4</sub> Cl.
Lordsburg, Cal. . . . .	Inches. 0-6	7.5 c. c. N/2 of each salt solution per 100 gm. of soil.	6.12	18.90	126.18	130.08	9.30	24.18	30.18	39.48
Do. . . . .	6-18	do. . . . .	4.2	27.72	119.70	123.60	10.50	30.00	31.02	47.22
Do. . . . .	18-30	do. . . . .	4.5	32.22	116.82	126.42	10.62	36.00	27.60	54.60
Do. . . . .	30-42	do. . . . .	4.35	37.95	120.30	159.75	11.10	43.50	29.70	60.45
Riverside, Cal. . . . .	0-6	do. . . . .	6.45	29.85	113.70	114.75	8.55	22.65	30.90	39.75
Do. . . . .	54-66	do. . . . .	4.05	36.30	110.55	112.50	7.95	41.55	29.40	56.55
Berwyn, Md. . . . .	0-6	do. . . . .	7.50	13.50	43.20					
Do. . . . .	6-18	do. . . . .	4.80	6.30	42.00					
Do. . . . .	18-30	do. . . . .	3.60	6.00	18.30					

Of the five salts used, aluminium chlorid gave the maximum effect, while the minimum effect was found with the sodium chlorid. The addition of an ammonium salt to a nonammoniacal salt gave a greater effect than when either of the salts was used alone. It is also observed that the increase in the solubility of calcium due to the addition of aluminium, sodium, or magnesium chlorid is fairly constant for the different depths, while the amount of calcium dissolved by the ammonium salts when used alone or in combination with other salts increases with the depth in the semiarid but not in the humid soils. This would seem to indicate a possible relation between the removal of calcium and the fixation of ammonia; but the data collected are too limited to warrant any definite conclusion at this time.

#### SUMMARY

(1) Many semiarid subsoils have the property of fixing large quantities of ammonia. Much of the ammonia fixed can not be removed by the ordinary methods for determining the ammonia content of the soils.

(2) Extracting the soil with 10 per cent hydrochloric acid gives approximately the same quantity of ammonia as distilling the soils with magnesium oxid.

(3) Anions apparently have little or no influence on the fixation of ammonia by soils.

(4) The ammoniacal nitrogen removed from duplicate samples of soil extracted with 10 per cent acid gives remarkably consistent results, while duplicate samples of soil distilled at atmospheric pressure with magnesium oxid frequently fail to give a satisfactory agreement.

(5) A large percentage of the ammonia added to semiarid soils and subsoils can not be recovered by boiling the soil with excessive amounts of caustic solutions.

(6) Boiling soils with 10 per cent hydrochloric acid removes practically all of the ammoniacal nitrogen from one soil studied, but less than 75 per cent was recovered from another soil.

(7) The fixation of ammonia by semiarid soils increases with depth. In this regard semiarid soils appear to differ from humid soils.

(8) The addition of ammonium salts in a concentrated solution results in greater fixation than when the same amount is added in a dilute solution.

(9) The fixation of ammonia by soils increases with the temperature.

(10) The fixation of ammonia by a soil is most rapid during the first few minutes, but the fixation process appears to continue for several days.

(11) Heating a soil for six hours at temperatures of 200° C. or above reduces its power of fixing ammonia.

(12) When small amounts of ammonium salts are added, the percentage of ammonia fixed remains constant. If increasing amounts of ammonia are added, a point is reached at which the percentage fixation becomes less, but the absolute fixation may continue to increase.

(13) Aluminium, iron, and potassium salts added to soils prior to the addition of ammonia reduce the ammonia-fixing power of the soils very decidedly.

Calcium, magnesium, and sodium salts added to the semiarid soils prior to the addition of the ammonia have little effect on the ammonia-fixing power of semiarid soils.

(14) The anions of aluminium, iron, potassium, calcium, sodium, or magnesium salts apparently have no influence on the action of these salts in reducing the ammonia-fixing power of semiarid soils.

(15) In semiarid soils the quantity of calcium brought into solution by ammonium chlorid increases with depth; when extracted with aluminium, sodium, or magnesium chlorid, the calcium brought into solution does not increase with the depth.



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## COMPOSITION OF CITRUS LEAVES AT VARIOUS STAGES OF MOTTLING

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A study of the soil factors influencing the mottling of Citrus leaves in southern California showed that the percentage of humus in the soils of the Citrus groves varied inversely with the mottling of the leaves, and this inverse relation showed a correlation of 67 per cent (2).<sup>1</sup> In another study it was found that organic matter, whether derived from stable manures, artificially produced manures from various green cover-crop materials, or by acid hydrolysis of sugar and hay material, attacked the soil minerals and liberated iron, calcium, magnesium, and phosphoric acid.<sup>2</sup> In another study on the mulched-basin system (3) it was found that on certain soil types this system caused a considerable diminution of leaf-mottling on orange trees (*Citrus aurantium*).

In view of these three facts it seemed possible that the mottling of Citrus leaves in southern California might possibly be due in part to a lack of supply of the mineral nutrients supposed to be most closely connected with the formation of chlorophyll. For this reason, Citrus leaves in various stages of mottling were collected and the iron, magnesium, calcium, and phosphoric-acid contents determined. The leaves in the various stages of mottling were collected from trees in the same grove, and in some cases from the same trees, so as to eliminate, as far as possible, the influence of variations in soil type.

Willstätter (11) has established the fact that the chlorophyll of all classes of plants contains magnesium and no other metal, and that the magnesium in the chlorophyll has an important part in the assimilation of carbon dioxide. Mameli (5, 6), in growing a number of plants in media lacking in magnesium, found that the plants were etiolated or pale green and the chloroplasts abnormal in form as well as in color. She further states that comparative analysis showed a smaller percentage of magnesium in chlorotic or discolored leaves or leaf parts than in normal portions of the same plant.

<sup>1</sup> Reference is made by number to "Literature cited," p. 166.

<sup>2</sup> Jensen, Charles A. Effect of decomposing organic matter on the solubility of certain inorganic constituents of the soil, particularly in its bearing on mottle-leaf of Citrus trees. *Not yet published.*

In another place (4) the same author reports that plants growing in media lacking manganese remained colorless or very pale and that by adding manganese in increasing proportions a corresponding increase in green color resulted.

Stoklasa, Brdlik, and Just (10), in repeating former work, reaffirm their earlier conclusion that phosphorus plays an important rôle in the production of chlorophyll. Their method of procedure was to extract the leaves with benzol and to determine the phosphorus in the extract.

One of the authors above cited, Brdlik (1), states that the physiological action in plants indicates a close relation between phosphorus and chlorophyll in the plant cell. He also states that alcohol and benzol extracts of green leaves show inorganic phosphorus as well as colorless phosphates, indicating that phosphorus plays a very important rôle in chlorophyll formation. It is his opinion that phosphorus plays as important a part in the physiological activity of the plant as magnesium or potassium.

Schryver (9) states that chlorophyll is a magnesium derivative from which the metal is eliminated readily by acids, but with great difficulty by alkalies.

Palladine (8) studied the influence of various carbohydrates on chlorophyll formation. Etiolated plants of *Vicia faba* and *Phaseolus vulgaris*, after being kept in the dark for 48 hours, were brought into the light. He states that the following substances favored the formation of chlorophyll: Saccharose, raffinose, glucose, fructose, maltose, glycerin, galactose, lactose, and dextrin. The following substances checked the formation of chlorophyll: Mannite, dulcitol, asparagin, urea, alcohol, ammonium chlorid, and quinic acid.

Mazé, Ruot, and Lemoigne (7) state that they produced chlorotic conditions in *Vicia narbonnensis* by adding 0.2 per cent of calcium carbonate to the culture medium, which condition was neutralized in three days by applying a few drops of iron nitrate to the leaves.

There are apparent contradictions in the findings of some of these authors. Willstätter states (11) that the chlorophyll of all plants contains magnesium and no other metal; Mameli (5, 6) that plants became chlorotic in the absence of manganese; Stoklasa et al. (10) that phosphorus plays an important part in the formation of chlorophyll.

It may be that the confusion on the subject is increased by mistaking an indirect influence of some of the above-mentioned elements for a direct one. For instance, iron was for a long time considered an essential constituent of chlorophyll, and, while it was later found not to be the case, iron is known to be quite essential in the formation of the green pigment in plants.

Palladine's results (8) on chlorophyll formation as influenced by various carbohydrates may be of great practical importance in orcharding. Especially may this be so in a system under so thorough control as the

mulched basin, when the decomposition products come into direct contact with the rootlets of the tree.

The mineral composition of Citrus leaves is subject to much variation, and the age of the leaf is an important factor to bear in mind when selecting leaves for comparative study. Since Citrus trees retain their leaves for two to three years, it is often difficult to determine the age of a certain leaf. Especially is this true of a leaf in the last stages of mottling, which, even when but 2 or 3 months old, often looks to be 2 or 3 years old.

It is also not a safe procedure to compare mottled leaves from a grove in a badly mottled condition with healthy leaves from another grove in a good condition, especially if the two groves are on different soil types.

In describing the mottled conditions of the leaves here discussed, the terms "first stage," "second stage," and so on to the "fifth stage" were used. These terms mean that stage 1 represents the first definite appearance of mottling, the mottled or chlorotic spots being limited to one to three small spots on each side of the midrib, the spots in this stage being usually confined to the upper part of the leaf. The fifth, or last, stage is the other extreme, which represents a leaf where only the midrib may retain a little chlorophyll or none at all. The stages 2, 3, and 4 simply represent conditions of increasing mottling and range between stages 1 and 5. The various stages are represented in color in one of the publications already cited (2).

In Table I are given the percentages of iron, calcium, magnesium, and phosphoric acid found in orange and lemon leaves in various stages of mottling, and also the relative distribution of these elements. The leaves were all of new spring growth and were collected on the fertilizer experiment plots of the University of California Citrus Experiment Station, Riverside. In each table the healthy leaves—that is, those with only a trace of mottling—are from trees on the manure plots, and the mottled leaves are from trees on the sodium-nitrate plots. These plots are side by side and represent very extreme tree conditions not only so far as leaf mottling is concerned but also as regards foliage density, fruiting, etc.

Considering the average composition of the entire leaves, the percentages of calcium, magnesium, and phosphoric acid increase as the mottling increases, in both the orange and lemon leaves. There is but one exception, that of the phosphoric acid in the last stage of mottling in the lemon leaves.

In most cases the ratio of the percentages of the determined elements in the leaves to those in the midribs diminishes slightly as mottling increases, though this variation is not very marked.

In each kind of leaves the average percentage of iron is highest in the healthy leaves and is least in stages 3 and 4.

TABLE I.—Composition of orange and lemon leaves in various states of mottling. Leaves collected on May 11, 1916

## ORANGE LEAVES

Part analyzed.	Stage of mottling.	Percentage distribution.				Percentage on dry substance.			
		Fe.	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .	Fe.	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .
Leaves minus mid-ribs.	Trace....	44	40	44	35	0.0096	2.17	0.266	0.437
Midribs alone.....	...do.....	44	36	27	31	.0092	1.98	.162	.396
Leaf stems.....	...do.....	10	24	29	34	.0020	1.35	.171	.433
Average of leaves and midribs.....						.0094	2.08	.214	.417
Leaves minus mid-ribs.	3-4	Tr.	34	40	38	Tr.	2.18	.300	.517
Midribs alone.....	3-4	45	39	35	30	.0022	2.52	.262	.411
Leaf stems.....	3-4	54	27	25	32	.0027	1.71	.190	.446
Average of leaves and midribs.....						.0011	2.35	.281	.464
Leaves minus mid-ribs.	Last.....	62	31	39	35	.0058	3.26	.342	.526
Midribs alone.....	...do.....	17	41	37	31	.0016	4.27	.324	.458
Leaf stems.....	...do.....	21	28	24	34	.0017	2.96	.202	.513
Average of leaves and midribs.....						.0037	3.77	.333	.492

## LEMON LEAVES

Leaves minus mid-ribs.	Trace....	45	41	38	37	0.0160	1.75	0.216	0.454
Midribs alone.....	...do.....	45	35	31	31	.0160	1.49	.173	.386
Leaf stems.....	...do.....	10	24	31	32	.0036	1.05	.175	.400
Average of leaves and midribs.....						.0160	1.62	.200	.420
Leaves minus mid-ribs.	3-4	52	44	33	38	.0120	2.26	.226	.657
Midribs alone.....	3-4	34	37	38	31	.0080	1.91	.259	.543
Leaf stems.....	3-4	14	19	29	31	.0033	0.98	.196	.535
Average of leaves and midribs.....						.0100	2.09	.243	.600
Leaves minus mid-ribs.	Last.....	30	29	40	22	.0132	3.42	.337	.248
Midribs alone.....	...do.....	42	39	40	33	.0180	4.58	.336	.370
Leaf stems.....	...do.....	28	32	20	45	.0120	3.78	.166	.513
Average of leaves and midribs.....						.0156	4.00	.387	.399



In the healthy leaves and usually also in the less mottled leaves of both orange and lemon the percentage of each mineral element is less in the midrib than in the mesophyll tissue. In leaves in the last stage of mottling, however, there is no definite uniformity in this respect, the midrib containing in some cases a higher and in some cases a lower percentage than the leaf proper. There does not appear to have been any difficulty about the transfer of these mineral elements from the conducting tissue to the mesophyll areas.

In most cases, regardless of the stage of mottling, the leaf stems contain less iron, calcium, and magnesium than either the midrib or mesophyll. The phosphoric acid is more evenly distributed in the three portions of the leaf.

Table II shows the results of analyses of orange leaves collected from a commercial grove near Riverside. There was decidedly less calcium and a little less magnesium in the leaves in the medium stage of mottling than in the healthy leaves or in the worst mottled leaves; otherwise, the remarks regarding the leaves discussed from Table I apply to those given in Table II.

TABLE II.—*Composition of orange leaves in various stages of mottling. Leaves collected on April 18, 1916*

Part analyzed.	Stage of mottling.	Percentage distribution.				Percentage on dry substance.			
		Fe.	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .	Fe.	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .
Leaves minus midribs.	None . . .	39	44	40	39	0.0126	2.88	0.261	0.436
Midribs alone. . . . .	...do. ....	39	36	33	33	.0126	2.35	.214	.368
Leaf stems. . . . .	...do. ....	22	20	27	28	.0075	1.35	.173	.308
Average of leaves and midribs. . . . .						.0126	2.62	.238	.402
Leaves minus midribs.	2-3	48	40	41	38	.0250	1.96	.242	.455
Midribs alone. . . . .	2-3	32	35	31	32	.0167	1.73	.183	.376
Leaf stems. . . . .	2-3	20	25	28	30	.0105	1.27	.159	.360
Average of leaves and midribs. . . . .						.0209	1.85	.213	.416
Leaves minus midribs.	3-4-5	26	34	37	31	.0185	3.22	.294	.400
Midribs alone. . . . .	3-4-5	29	42	36	35	.0200	4.05	.285	.450
Leaf stems. . . . .	3-4-5	45	24	27	34	.0316	2.28	.220	.430
Average of leaves and midribs. . . . .						.0193	3.64	.290	.425

The leaves reported in Table III were collected on January 3, 1916, in commercial groves near Riverside. These leaves, except the last set given, were collected from the same grove; usually the healthy and mottled leaves were taken from the same tree branches, where both

healthy and mottled leaves of approximately the same age could be obtained.

As in the preceding determinations, the worst mottled leaves have the highest percentage of the mineral elements under investigation; and the percentage distribution shows no consistent differences between the conducting tissue and the mesophyll areas.

TABLE III.—Analysis of orange leaves in various stages of mottling. Leaves collected on January 3, 1916

Part analyzed.	Stage of mottling.	Percentage distribution.			Percentage on dry substance.		
		Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .
Leaf minus midrib.....	None.....	37	32	32	2.87	0.332	0.500
Midribs alone.....	do.....	39	39	35	3.07	.401	.482
Leaf stems.....	do.....	24	29	29	1.85	.294	.400
Average of leaves and midribs.....					2.97	.367	.491
Leaf minus midrib.....	2-4	50	44	49	2.28	.275	.531
Midribs alone.....	2-4	50	56	51	2.30	.323	.551
Average of leaves and midribs.....					2.29	.299	.541
Leaf minus midrib.....	4-5	43	51	40	1.78	.267	.563
Midribs alone.....	4-5	57	49	60	2.39	.261	.835
Average of leaves and midribs.....					2.09	.264	.699
Leaf minus midrib.....	Last (leaves all yellow, midribs green).	30	37	32	2.66	.344	.530
Midribs alone.....	do.....	41	32	39	3.67	.298	.646
Leaf stems.....	do.....	29	31	29	2.62	.294	.473
Average of leaves and midribs.....					3.17	.321	.588
Leaves.....	Very spotted; yellow spots.	28	27	23	2.69	.204	.403
Do.....	Green spots; mostly veins.	34	42	31	3.24	.319	.553
Midribs.....	do.....	38	31	46	3.68	.242	.796
Average of leaves and midribs.....					2.97	.262	.483

The last set of leaves given in Table III was collected in another grove, where the mottled appearance of the leaves was much accentuated, the boundary lines between the chlorotic tissue and the green tissue being very sharp. The mottled spots were cut out and these and the remaining green parts, mostly veins, were separately analyzed. The green areas contain decidedly more of the elements determined than the yellow spots,

but there does not seem to have been any definite accumulation of these elements in the midribs, with the exception of phosphoric acid. The average percentage of the various elements in the whole leaf is about the same as in the other leaves reported in Table III.

Table IV shows a comparison of the composition of old and new leaves of grapefruit (*Citrus decumana*). The difference in the calcium and the magnesium contents is striking, though the amount of phosphoric acid is about the same in the old and new leaves. The results show the importance in a study of this kind of securing leaves of as nearly the same stage of development as possible.

TABLE IV.—Analysis of old and new grapefruit leaves. Collected on January 3, 1916.

Part analyzed.	Description.	Percentage distribution.			Percentage on dry substance.		
		Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .
Leaves minus midribs...	Old leaves under the new leaves given below.	49	48	52	5.06	0.425	0.455
Midribs alone.....	.....do.....	51	52	48	5.24	.471	.416
Average.....	.....				5.15	.448	.436
Leaves minus midribs...	New leaves above the old leaves given above.	47	56	57	2.13	.332	.560
Midribs alone.....	.....do.....	53	44	43	2.41	.260	.431
Average.....	.....				2.27	.296	.496

Table V shows the analyses of leaves from a privet plant (*Ligustrum aurea*) growing in White Park, Riverside, Cal. A number of branches produced in part leaves which were light yellow or almost white in color. The percentage of calcium was found to be considerably greater in the green leaves than in the yellow leaves. The percentage of magnesium was greater in the green leaves, while the percentage of iron and of phosphoric acid was greater in the yellow leaves, indicating that the absence of chlorophyll is not likely to be due to lack of iron or phosphoric acid. The leaf stems of the yellow leaves contain more of each of the elements determined than the leaf stems of the green leaves, which might be interpreted to indicate that the transfer of mineral nutrients did not take place as freely from the leaf stems in the yellow branches as in the green ones.

In the last set of analyses of privet leaves given in Table V, the yellow margins also contained more iron and phosphoric acid than the green midribs, but about one-half as much calcium and a little less magnesium. Of the two elements necessary for chlorophyll formation, iron and magnesium, iron is present in larger amounts in the yellow

leaves than in the green leaves, and the percentage of magnesium is almost as great.

TABLE V.—*Analysis of normal green leaves and of yellow leaves of privet. Collected on July 1, 1916*

Part analyzed.	Description.	Percentage on dry substance.			
		Fe.	Ca.	Mg.	P <sub>2</sub> O <sub>5</sub> .
Entire leaf.....	Normal green leaves (entire leaf).	0.0225	2.03	0.250	0.481
Leaf stems of above.....	do.....	.0108	.844	.131	.326
Yellow leaves.....	Entire leaf yellow, including midrib.	.0388	1.035	.196	1.165
Leaf stems of above.....	do.....	.0386	1.350	.174	.522
Yellow leaf margins..	Green midrib, yellow leaf spread.	.0312	.806	.221	1.305
Green midribs of above.	do.....	.0242	1.78	.259	.815
Leaf stems of above.....	do.....	.0197	.985	.148	.448
Average of yellow margins and green midribs.		.0277	1.293	.240	1.060

#### SUMMARY

Previous studies by this office in southern California have shown that the percentage of mottling of the Citrus leaves varied inversely with the humus content of soils in Citrus groves; that decomposing organic matter increases the amounts of soluble salts in the soil; and that a system of basin mulching in Citrus groves, especially on certain soil types, has produced an improvement in tree growth and fruit setting in comparison with the furrow system of irrigation and surface cultivation.

The purpose of the study here reported was to see if mottled Citrus leaves showed a deficiency of the mineral elements directly affecting chlorophyll formation. If this were the case, better leaf growth on Citrus trees in orchards well supplied with active organic matter might be associated with the greater amount of soluble mineral plant food in a soil well supplied with decomposing organic matter.

It was found that orange and lemon leaves very badly mottled contained higher percentages of iron, calcium, magnesium, and phosphoric acid than healthy leaves, the average percentage of the entire leaf being considered.

The leaves in the medium stages of mottling sometimes contained more and sometimes less of these elements than healthy leaves.

In nearly all cases the midribs of the healthy leaves contained less of the above-mentioned elements than the mesophyll tissue. In badly mottled leaves the midribs contained a higher percentage of calcium

than the mesophyll tissue, usually as much magnesium, and usually more phosphoric acid.

With very few minor exceptions, the leaf stems contained less iron, calcium, and magnesium than either the midrib or mesophyll area in both healthy and mottled leaves. The percentages of calcium, magnesium, and phosphoric acid, however, increased in the leaf stems of badly mottled Citrus leaves, but usually not in the medium mottled leaves.

Old leaves contained higher percentages of calcium and magnesium than new leaves not fully grown.

In all the Citrus leaves analyzed, the phosphoric acid was quite uniformly distributed in the midribs, the mesophyll tissue, and the leaf stems (regardless of age or stage of mottling), indicating that phosphoric acid is early and freely transferred through the conducting tissue to the mesophyll areas.

Sharply outlined yellow spots in the mesophyll areas of orange leaves contained less calcium, magnesium, and phosphoric acid than the green parts (mostly veins) of the leaves.

Green leaves and the green parts of spotted leaves of the golden privet contained about twice as much calcium and appreciably more magnesium than the yellow leaves. Yellow leaves and the yellow parts of spotted leaves contained more iron than and about 2.5 times as much phosphoric acid as the green leaves or green parts of spotted leaves.

Leaf stems of green privet leaves contained lower percentages of iron, calcium, magnesium, and phosphoric acid than the leaves.

Leaf stems of yellow privet leaves contained about one-half as much phosphoric acid as the leaves; the percentages of iron and magnesium were about the same, while the leaf stems contained more calcium than the leaves. The leaf stems of yellow privet leaves contained higher percentages of calcium and magnesium than the leaf stems of green privet leaves.

Judged by a comparison of the average percentages of the inorganic elements determined in healthy Citrus leaves and in leaves in the medium stages of mottling, the data obtained did not show that the initial mottling could be accounted for by deficiency in the transfer of the iron, calcium, magnesium, and phosphoric acid from the conducting system of the leaf stem and midrib to the mesophyll tissue.

On the other hand, sharply localized yellow areas in old orange leaves contained less of these elements than the adjoining green areas (mostly veins), but whether that relation obtained in the initial stage of mottling was not determined.

In very badly mottled Citrus leaves there was in general an increase in the percentage of these elements in the conducting tissues, including the leaf stems, indicating difficulty in their transfer to the mesophyll tissues in very advanced stages of mottling, probably because the leaf had become functionless.



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# EFFECTS OF MUSCULAR EXERCISE AND THE HEAT OF THE SUN ON THE BLOOD AND BODY TEMPERATURE OF NORMAL PIGS<sup>1</sup>

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## INTRODUCTION

This work was carried out, in part, along with our studies of the blood of normal resting pigs. The work first suggested itself on June 25, 1915. We had just started our work on normal adult pig's blood, and had confined in a small outside pen several pigs. These pigs had been quiet, but it was noticed that a certain red sow was forced to lie directly in the sun. This animal was breathing rapidly, but was thought to be normal. On taking her temperature it was found to be 106° F., and the blood clotted at 15 seconds (about 60 seconds being normal). By permitting the animal to lie in the shade the body temperature soon returned to normal, as did the clotting time of the blood. A differential count of the leucocytes at this time showed an unusually high percentage of polymorphs, and an unusually low percentage of lymphocytes. Although we were familiar with the fact that muscular exercise causes these changes in the blood of man, we were surprised to learn that heat could cause similar changes in the blood of the pig. It was therefore decided to continue these studies of the pig.

Some time after this experience several papers appeared in the literature dealing with the effects of muscular exercise upon the blood of man; and, in carefully going over the literature, it was observed that the cause of the various changes in the blood of man resulting from muscular exercise was unsettled. It is therefore hoped that the results of this work on the pig may throw a little light on these phenomena.

## METHODS OF STUDY

The animals used in this work were normal hogs weighing about 100 pounds. They had previously been in a resting state for a couple of days. They were taken from their pens, placed in a special hog crate, and the various determinations made. The results of these examinations were added to our data concerning the examination of normal

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<sup>2</sup> I wish to take this opportunity to express my great appreciation of the valuable assistance rendered by my associate, Mr. Arthur L. Anderson, in carrying out this work.

hog's blood. The animals were then removed from the crate and exercised for a certain length of time, or placed in a small wire pen, exposing them to the sun according to the series to which they belonged. Some pigs were very easy to exercise, and would drive freely for several minutes and would begin to show symptoms of fatigue and a high body temperature 10 to 15 minutes after the exercise had started. Many pigs, on the other hand, were difficult to drive; it is difficult to get them to move at a faster gait than a walk, and with such animals it usually requires about one-half hour of exercise before they show fatigue and an increase in body temperature. Immediately after the exercise or sun exposure the various blood examinations were again determined.

In a number of animals dry spreads were made at certain intervals, covering a period of 24 to 48 hours or longer, following the exercise or exposure to the sun. In this manner the leucocytic curve was established.

The methods of technic were similar to those used in our studies on the blood of normal pigs. The temperature throughout each experiment was recorded.

#### I.—EFFECTS OF MUSCULAR EXERCISE

##### A.—EFFECTS ON THE RED CORPUSCLES

It is well established that exercise in man causes an increase in the number of red corpuscles. Tornow<sup>1</sup> found in experiments on soldiers after long marches an increase of about 9 per cent in the number of red corpuscles. Hasselbalch and Heyerdahl (2) in two experiments found an increase of 13 and 17 per cent. Boothby and Berry (2) found an average increase of 19 per cent, but the increase occurred only when sweating was marked. Willebrand (5) found in 12 young men during gymnastic work an increase which varied between 2.9 and 23.4 per cent; and the length of time consumed in returning to normal was quite variable. Zuntz and Schumberg (5, 7), in a study of German soldiers during marches, obtained an average increase of 9 per cent. Hawk (5), in his studies of college athletes, found an increase from 7.3 to 26.7 per cent. Schneider and Havens (7), in their studies on college athletes, found an increase which varied between 3.2 and 22.8 per cent.

In animals Cohnstein and Zuntz (5) examined the blood of rabbits, after subjecting the animals to a systematic harassing for several minutes. They found a slight decrease in the number of red corpuscles and an accompanying leucytosis.

In the blood of 15 pigs studied in this laboratory there were practically no changes in the number of red corpuscles (Table I). There were slight individual variations in the counts before and after exercise; in some cases there was an increase and in others a decrease following the exercise. These variations, however, were no different than those found in

<sup>1</sup> Reference is made by number to "Literature cited," p. 182.

normal resting pigs (see "Control experiments"). The average count before and after exercise showed 6,098,733 red cells per cubic millimeter before and 6,053,666 after exercise.

TABLE I.—Effects of muscular exercise on 15 normal pigs

BEFORE EXERCISE														
Animal No.	Weight of animal.	Condition of animal.	Color and sex of animal.	Temperature of animal.	Blood.					Differential count.				
					Clotting time.	Hemoglobin.	Specific gravity.	Number of erythrocytes.	Number of leucocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Masts.
	Lbs.			°F.	Sec.	%				%	%	%	%	%
6981.....	74	Good..	Black and white female.	102.8	65	70	1.070	5,640,000	14,000	50.65	46.75	1.3	0.52	0.78
7073.....	101	do..	White male..	99.3	70	70	1.063	5,156,000	33,000	42.4	51.42	.16	5.52	.47
7015.....	101	do..	Female.....	102.4	70	78	1.070	6,831,800	14,000	60.92	25.73	.0	10.0	3.34
7071.....	108	do..	Red female..	102.4	70	65	1.069	6,196,000	17,000	50.97	46.09	.42	2.37	.14
7160.....	85	do..	White male..	101.6	55	70	1.064	5,272,000	19,000	42.25	55.36	1.21	.0	.17
7162.....	88	do..	White female.	103.2	40	70	1.070	5,456,000	19,000	53.09	43.43	.58	2.31	.58
7216.....	111	do..	Black male..	103.6	70	70	1.066	5,320,000	18,000	56.92	35.86	.19	5.88	1.14
7217.....	102	do..	Black female.	102.8	35	70	1.071	6,380,000	15,000	62.83	28.32	.36	5.13	3.37
7273.....	105	do..	Black male..	102.4	55	70	1.067	5,960,000	15,000	70.62	24.48	.19	4.33	.37
7271.....	105	do..	Black female.	103.6	35	70	1.070	6,104,200	18,000	68.43	25.42	.34	4.26	1.53
7279.....	120	do..	Brown female	103.3	30	112	1.070	7,580,000	19,000	59.17	37.04	1.54	1.37	.85
7278.....	110	do..	Black and white male.	101.8	60	102	1.066	5,976,000	20,000	57.05	26.61	1.4	13.11	2.09
7276.....	110	do..	White female.	102.8	75	112	1.069	6,217,000	20,000	.....	.....	.....	.....	.....
6308.....	110	do..	do.....	102.2	60	115	1.071	7,568,000	17,000	58.88	40.18	.37	.56	.0
7272.....	130	do..	Black female.	103.5	20	100	1.066	5,624,000	28,000	59.98	38.61	.38	4.82	.19
Average...	104	.....	.....	102.5	54	83	1.068	6,098,733	19,066	56.72	37.52	.60	4.30	1.07
AFTER EXERCISE														
Animal No.	Length of exercise of animal.	Condition of animal.	Temperature of animal.	Blood.					Differential count.					
				Clotting time.	Hemoglobin.	Specific gravity.	Number of erythrocytes.	Number of leucocytes.	Lymphocytes.	Polymorphs.	Mononuclears.	Eosinophiles.	Masts.	
	Min		°F.	Sec.	%				%	%	%	%	%	
6981.....	30	Rapid breathing.....	107.0	15	70	1.068	6,218,000	31,000	31.11	66.66	2.0	0.0	0.22	
7073.....	30	do.....	107.0	25	80	1.068	5,012,000	33,000	34.38	65.04	.11	.23	.23	
7015.....	30	do.....	106.9	20	86	1.072	5,896,000	20,000	37.24	58.66	.93	1.86	1.3	
7071.....	30	do.....	109.4	20	77	1.070	6,040,000	30,000	27.46	70.74	.96	.55	.27	
7160.....	30	do.....	107.6	20	67	1.062	5,140,000	25,000	28.46	70.23	1.0	.33	.33	
7162.....	15	do.....	106.4	30	75	1.070	5,664,000	19,000	44.75	52.47	.2	1.18	1.38	
7216.....	15	Not much change.....	105.9	30	75	1.065	5,350,000	16,000	57.57	34.78	.27	5.45	1.91	
7217.....	30	Rapid breathing.....	107.8	25	70	1.068	6,232,000	25,000	47.58	48.40	.48	2.41	1.13	
7273.....	15	do.....	106.4	20	78	1.066	6,120,000	15,000	56.74	39.70	.97	2.43	.16	
7271.....	25	do.....	107.2	20	78	1.070	5,912,000	24,000	57.07	39.56	.5	1.34	1.34	
7279.....	15	do.....	108.9	20	114	1.069	7,600,000	22,000	49.83	48.68	.82	.16	.49	
7278.....	15	Not much change.....	105.0	30	100	1.066	5,856,000	20,000	53.90	38.05	.53	5.67	1.24	
7276.....	15	Rapid breathing.....	107.7	40	110	1.068	6,475,000	22,000	.....	.....	.....	.....	.....	
6308.....	15	No change.....	103.6	30	112	1.071	7,826,000	16,000	40.81	53.46	1.42	3.26	1.02	
7272.....	15	Rapid breathing.....	107.8	15	100	1.066	5,464,000	22,000	46.74	50.09	.0	2.63	.52	
Average.....	.....	.....	106.9	24	86.83	1.068	6,053,666	22,666	43.83	52.61	.72	1.96	.82	

Several investigators, working with the blood of man, have found a leucocytosis following muscular exercise. Hawk (5) cites three investigations in which a leucocytosis was found after muscular work, the number of leucocytes varying from 11,400 to 22,200 (normal, 8,000 to

## B.—EFFECTS ON THE LEUCOCYTES

10,000). Zuntz and Schumberg (5, 7) found an increase of 43 per cent in the number of leucocytes in soldiers, following marches. Hawk, in his college athletes, found an increase of from 21 to 104.4 per cent, with an average of 57.0 per cent. Schneider and Havens (7) found an increase which varied from 13.8 to 130.2 per cent and returned to normal in a very short time (30 to 45 minutes).

In animals Cohnstein and Zuntz (5) found a leucocytosis in rabbits, following muscular exercise. In pigs we have found, on an average, an increase of 18.88 per cent. This increase, however, was not uniform; and in some cases it was lower after exercise than before (for explanation, see "Discussion of results" and "Control experiments").

## I.—CHANGES IN DIFFERENTIAL COUNTS

Larrabee (3, 5) not only found a leucocytosis in the blood of the long-distance runners which he examined but he also found the polymorphs to be increased. The eosinophiles were absent in three cases and much reduced in the fourth. The number of transitionals was increased. Zuntz and Schumberg (5, 7) obtained an increase of polymorphs from 6 to 11 per cent, and a decrease in the lymphocytes from 3 to 17 per cent. Schneider and Havens (7) found an increase of 9 to 45 per cent in polymorphs, and a decrease of 14 to 55 per cent in the lymphocytes. They found no definite change in the proportions of the various kinds of leucocytes at the close of the exertion, but slowly thereafter and throughout a period of from 1 to 2 hours the polymorphs increased and the lymphocyte index (including lymphocytes, mononuclears, and transitionals) decreased. They found that the normal proportions returned after about 2 to 4 hours. They state that the changes in the differential count continued long after the normal number of leucocytes had been returned. Burrows (7), on the other hand, in a study of a single case, found that exercise decreased the polymorphs and increased the lymphocytes.

In pigs we have found a condition similar to that reported by Larrabee, Zuntz, and Schumberg and Schneider and Havens for man, except that the normal proportions did not return until after a much longer period. Differential counts of the leucocytes indicate a slow destruction of the lymphocytes of the blood following muscular exercise and an increase of the polymorphs.

In 15 experiments in which the examinations were made shortly after the exercise there was an average decrease of 12.61 per cent of lymphocytes, an average increase of 15.13 per cent of polymorphs, an average decrease of 2.34 per cent of eosinophiles, a very slight increase in the mononuclears, and a slight decrease in the masts.



## 2.—DIFFERENTIAL CURVE

In five experiments an attempt was made to follow these changes in the differential count until the normal proportions returned. Spreads were made at intervals of a few hours and covering a period of 24 to 48 hours.

(a) LYMPHOCYTE CURVE.—The lymphocytes were decreased, the height of the reaction occurring from two to seven hours after exercise, with an average of four hours. At this time they were decreased 29.43 to 39.77 per cent, the average being 32.75 per cent. After the height of the reaction there was a gradual return to the normal proportions, which took place after 36 to 48 hours.

(b) POLYMORPHONUCLEAR CURVE.—The polymorphs were increased, the height of the reaction occurring from two to seven hours after exercise, and the average high point of the reaction was four hours after exercise. At this time they were increased 33.79 to 44.77 per cent, with an average increase of 39.88 per cent for the entire five animals. Following the height of the reaction the normal proportions gradually returned, and the time required varied between 36 and 48 hours.

(c) EOSINOPHILE CURVE.—The eosinophile curve varied from that found in the lymphocytes and polymorphs in that these cells showed a double curve. Shortly after the exercise (4.5 hours) the eosinophiles dropped to zero, or nearly zero; following this there was a gradual increase until these cells became much higher than the percentage normally found. The height of this latter curve was reached about 24 hours after exercise. Following this, the normal proportion gradually returned, and the count was again normal at 30 hours or longer.

Table II details the changes in the eosinophile count in five experiments.

TABLE II.—*Changes in the percentages of eosinophiles covering a period of 48 hours following the muscular exercise of pigs*

Animal No.	Normal percentage.	Lowest percent age.	Difference.	Hours after exercise when percentage is lowest.	Highest percentage.	Hours after exercise when percentage is highest.	Hours after exercise when return to normal occurs.
			<i>Per cent.</i>				
1.....	0.56	0.00	0.56	4.5	11.01	24	Not established.
2.....	3.00	.58	3.42	4.5	9.68	24	Do.
3.....	1.37	.00	1.37	4.5	11.88	24	Do.
4.....	13.11	.17	12.94	7.0	15.63	24	Do.
5.....	4.82	.00	4.82	2.0	11.02	29	About 30 hours.

(d) MAST CURVE.—The behavior of the mast cells was very similar to that of the eosinophiles, and the curve was irregularly parallel with the eosinophile curve.

(e) MONONUCLEAR CURVE.—The mononuclears were decreased at first, and later returned to normal. Their behavior was quite similar to the lymphocytes. Figure 1 shows in detail the various curves for one animal following muscular exercise.

#### C.—EFFECTS ON HEMOGLOBIN

Boothby and Berry (2) found an increase in the percentage of hemoglobin, and this increase occurred only when the red corpuscles were

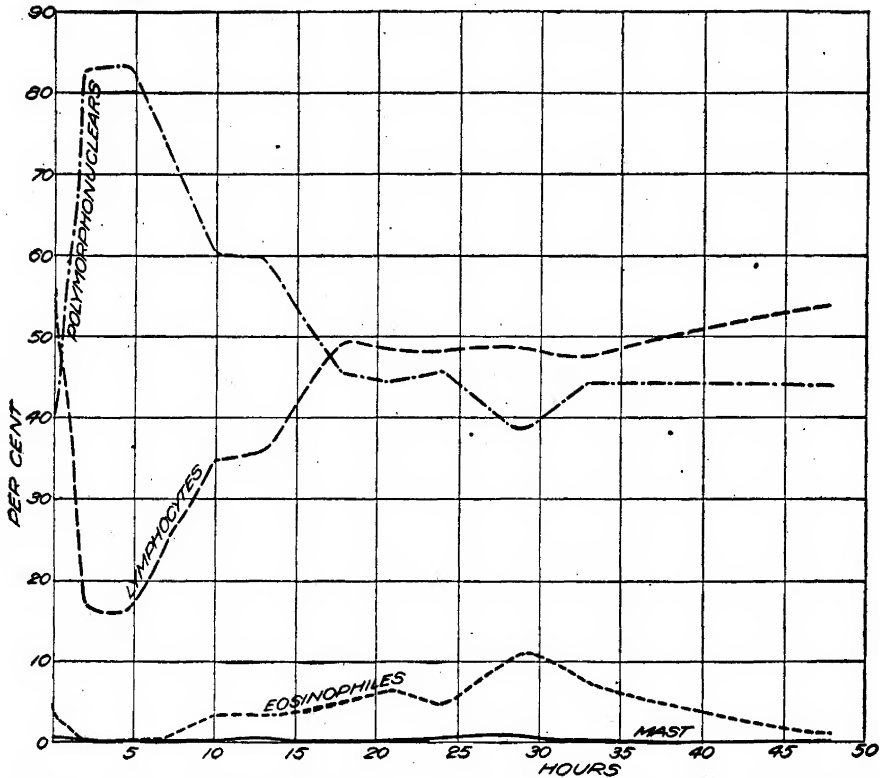


FIG. 1.—Graphs showing the detail of leucocytic changes in the blood of one pig following muscular exercise.

increased. These changes did not occur unless there was marked perspiration. These workers found the increase to vary between 7 and 11 per cent. Schneider and Havens (7) found the changes in hemoglobin, on the whole, proportionate, but not always equal, to the increase in the number of red corpuscles. They found the increase to vary between 3.5 and 10.9 per cent.

In the pig we have found an average increase of 3.13 per cent after exercise. The increase was not consistent. The percentage was found to be higher in 8 cases, lower in 4, and even in 3.

## D.—EFFECTS ON SPECIFIC GRAVITY

The specific gravity of the blood of man in muscular activity has been studied by Jones (6). He found that it usually varied directly with the red corpuscles. He reports, however, that gentle exercise is accompanied by a fall, while the more prolonged or violent forms of exercises are accompanied by a rise in the specific gravity. Zuntz and Schumberg (5, 7) constantly obtained an increase in the specific gravity of the blood. Schneider and Havens (7) found that exercise invariably caused a rise.

In pigs we did not find an increase in specific gravity when the entire experiment was considered.

## E.—EFFECTS ON CLOTTING TIME

As one would naturally expect, the clotting time was greatly increased. The clotting time seems to be varied in a rough way with the body temperature of the pig.

## CONTROL EXPERIMENTS

Several control experiments were carried out, whereby blood counts were made on normal resting pigs at various intervals. In making the wet counts the same pipette was used in each experiment, and the blood samples were obtained from a fresh vein in either ear. The dry spreads were obtained in a similar manner—that is, a fresh clean vein was used in making each spread.

Results of this work showed that, although in some pigs the results are quite uniform both in the wet and dry counts, it is often found that the blood of the pig is subject to considerable variation at different times.

In some pigs the wet counts were very uniform and showed no variations other than normal. In others there was sometimes as much as 1,000,000 red corpuscles per cubic millimeter difference in two examinations on the same animal. The differential count of leucocytes also showed similar results. In some pigs the differential count covering a period of 24 hours was quite uniform and showed very slight changes, varying between zero and 2 to 3 per cent. In others, however, there was marked variation. The individual leucocytes varied at different times, and the greatest variation occurred in the polymorphs and lymphocytes. Five experiments dealing with the differential counts were made in normal resting pigs and covering a period of 24 hours (12 counts made).

## II.—EFFECTS OF HEAT OF SUN

In this series of five experiments on the effects of the sun's heat only the changes in the leucocytes were studied. The differential curve for a period of 36 to 72 hours was worked out. The results in general were quite similar to those obtained in the animals which were given muscular exercise.

(a) LYMPHOCYTE CURVE.—The lymphocytes were decreased in number, the height of the reduction occurred from 4.5 to 7 hours after being placed in the sun, and the average for the five experiments was 6 hours. At this time they were decreased 5.06 to 17.01 per cent, the average being 11.48 per cent. After this there was a gradual return to the normal proportions, which took place after 15 to 48 hours, the average being 29 hours. The greatest change required the longest time to return to normal.

(b) POLYMORPHONUCLEAR CURVE.—The polymorphs were increased in numbers, the height of the reaction occurred from 4.5 to 7 hours after being placed in the sun, and the average for five experiments was 6 hours. At this time they were increased 3.79 to 21.63 per cent above the normal, the average increase being 11.4 per cent. After the height of the reaction there was an irregular return to the normal, which took place between 15 and 72 hours or more. The lowest change required the least time to return to normal.

(c) MONONUCLEAR CURVE.—There was a slight decrease in the number of mononuclears. The height of the reaction occurred about 6 hours after being placed in the sun. Following this there was a gradual increase to the normal proportions. This increase was quite irregular. In general, the behavior of these cells was quite similar to the lymphocytes.

(d) EOSINOPHILE CURVE.—The behavior of the eosinophiles was, in general, very similar to this class of cells following muscular exercise. If the normal percentage of these cells was rather high, there was always a decrease, the height of the reaction occurring about 5 to 7 hours after being placed in the sun. Following this there was a gradual increase in the number of these cells, the increase running far beyond the normal percentage. The height of this increase was attained from 13 to 27 hours after being placed in the sun. Then there was a gradual return to the normal percentage. If the normal percentage was low, the first decrease did not occur, but there was a gradual increase until about 13 to 27 hours after being placed in the sun, when the height of the reaction occurred. After this there was a gradual return to the normal proportions.

TABLE IV.—Details of the changes in the percentages of eosinophiles covering a period of 72 hours following exposure of pigs to the sun

Animal No.	Normal percentage.	Lowest percentage.	Difference.	Hours after exposure in sun when percentage is lowest.	Highest increase in percentage.	Hours after exposure in sun when increase in percentage is highest.	Hours after exposure in sun when return to normal occurs.
1.....	0.19	Gradual increase.	<i>Per cent.</i> Gradual increase.	.....	5.00	13	Not established.
2.....	4.95	0.36	4.59	5	9.19	31	60 hours.
3.....	1.37	Gradual increase.	Gradual increase.	.....	5.12	27	Not established.
4.....	1.57	...do.....	...do.....	.....	4.98	27	48 hours.
5.....	2.14	.19	1.95	7.	7.88	27	60 hours.

(e) Table IV shows in detail the changes in the percentages of eosinophiles at the turning points of the curve.

MAST CURVE.—The behavior of the mast cells was very similar to that of the eosinophiles, and the curve was irregularly parallel with the eosinophile curve.

Figure 2 shows in detail the various curves for one animal following exposure to the sun.

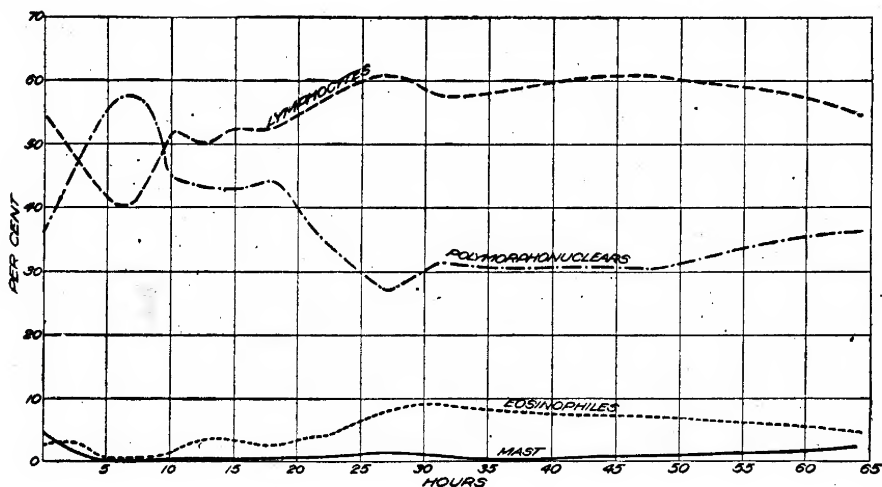


FIG. 2.—Graphs showing the detail of leucocytic changes in the blood of one pig following sun exposure.

### III.—EFFECTS OF MUSCULAR EXERCISE AND HEAT OF THE SUN ON BODY TEMPERATURE

The body temperature of animals is known to show more variations than the body temperature of man. In animals many external conditions cause variations in body temperatures. Lee (1) has called attention to recent findings of the New York Commission on Ventilation with respect



to an apparent relationship between the body temperature of man and the temperature of his environment, even under ordinary conditions of living. It was found, for example, that during summer months the rectal temperature at 8 a. m. of people living in their own homes was conditioned by the average atmospheric temperature of the preceding night. If the temperature had been warm, the body temperature in the morning was high; if cool, the body temperature was low. The variation was about  $1^{\circ}$  F. for 20 degrees of atmospheric temperature. The body temperature was lowered by confinement in an atmosphere of  $68^{\circ}$  and 50 per cent relative humidity and raised by confinement at  $75^{\circ}$  with the same humidity, or still more by  $86^{\circ}$  with 80 per cent humidity. The actual body temperatures found at these stages, respectively, were  $98^{\circ}$ ,  $98.5^{\circ}$ , and  $99.3^{\circ}$ .

In extreme atmospheric conditions greater elevations of temperature are known to occur (1). A stay of about three hours in an atmosphere averaging  $104.7^{\circ}$  in temperature and 95 per cent relative humidity may produce a rise of several degrees in the body temperature of a normal adult man.

Schroeder and Mohler (8), in their bulletin dealing with the tuberculin test of hogs, state that, when the temperature of a number of hogs is compared, the difference found is of such magnitude that they are at a loss to conclude what should be regarded as normal. Other than stating that the normal variations that occur in individual hogs are very great, they do not give any figures. These workers have also found that fat hogs have a higher temperature than lean ones and that a higher temperature induced by exercise or some other temporary cause persists longer in fat than in lean hogs.

We have not collected any large amount of data concerning temperature variations in normal pigs other than the temperature records which accompanied our blood experiments. These experiments, however, very clearly demonstrated the extreme variability in the temperature of pigs.

Merely being in the direct rays of the sun will cause a marked increase in the body temperature, the increase, of course, varying with the atmospheric temperature and relative humidity. On a hot day a normal pig exposed to the direct rays of the sun may show a temperature as high as  $106^{\circ}$  to  $108^{\circ}$  F.

The handling of or working among a group of pigs for only a few minutes can cause a marked increase in their body temperatures. For example, a black sow weighing 102 pounds had been quiet in a small pen for two days. Her rectal temperature was then  $102.8^{\circ}$ . She was then picked up and placed in a hog crate, the operation requiring only a few minutes, but it caused a rise in the rectal temperature to  $104^{\circ}$ .

A black sow weighing 130 pounds and in good condition, but not fat, had been confined for several days in a small pen. Before taking her out

of the pen her temperature was  $102.3^{\circ}$ . She was driven slowly across a 50-foot lot and placed in a crate, her temperature at this time being  $103.5^{\circ}$ . She was in the crate for 30 minutes, and during this time fought some. At the end of this period her temperature was  $105.8^{\circ}$ . She was then exercised for 15 minutes at a slow pace (could not get her to go much faster than a walk), and following this exercise her temperature was  $107.8^{\circ}$ . She was then placed in the crate again, the various blood examinations were made, and just before taking her out of the crate she showed a temperature of  $108.1^{\circ}$  and was dyspneic.

The mere handling of the animals, as holding for examination or placing them in a crate, especially if they offer any resistance, will cause an increase in the temperature of a degree or two, even when the temperature is already high. For example, a black male weighing 105 pounds showed a pen temperature of  $102.4^{\circ}$ . After placing him in the crate the temperature was increased to  $104^{\circ}$ . The animal was still just before removing him from the crate, and the temperature had been lowered to  $103^{\circ}$ . The animal was then exercised for 15 minutes (on a rather cool day), and after the exercise the temperature was increased to  $105.6^{\circ}$ . After placing the animal in the crate, the temperature was increased to  $106.4^{\circ}$ , and just before removing from the crate it had been lowered to  $106.2^{\circ}$ . Very slight struggling in the crate will keep an already high temperature elevated and tend to increase a low one.

Although it was not our intention to see how high the temperature would go, it might, however, be worthy of recording that the highest temperature after exercise was noted in a red sow weighing 108 pounds. The animal was in very good condition. Before exercise the sow showed a temperature of  $102.4^{\circ}$ . After exercising for one-half hour at a slow gait, the temperature was increased to  $109.4^{\circ}$ .

After exercising and obtaining the blood samples, if the changes were not to be followed further, the animals were given a cold shower bath, which quickly lowered the temperature and which the animals thoroughly enjoyed.

If allowed to remain quiet, the temperatures would return to normal in one-half to one hour.

## DISCUSSION OF RESULTS

### CHANGES IN THE ERYTHROCYTES AFTER MUSCULAR EXERCISE

Muscular exercise does not cause an increase in the number of red corpuscles in the blood of the pig. This is difficult to explain. It is possible that there was an increase following the exercise; but the normal proportions had returned before the count was made, although such an explanation seems hardly plausible in light of the work of Zuntz and Schumberg (5, 7), who found an increase in the number of red corpuscles in soldiers after long marches. Schneider and Havens (7) found the height of the erythrocyte curve was not reached until 75

minutes after an 0.8 mile run, and the normal proportions did not return for 2 hours. In pigs our counts were made in all cases quite within this time.

Hawk (5) advances six possible explanations for the increase of erythrocytes in exercise. These are (1) the production of new corpuscles; (2) concentration of the blood through increased urine formation and copious sweating; (3) concentration of the blood through increased evaporation in the lungs; (4) concentration of the blood through vaso-motor contraction and rise in blood pressure; (5) sudden passage into the circulating blood of a large number of cells lying in various parts of the body and inactive before the time of muscular exercise; and (6) passage of fluid from the blood to active muscles.

Hawk concludes that the number of red corpuscles produced by muscular exertion is due primarily to the passage into the circulating blood of a large number of cells lying in various parts of the body and inactive before the time of the muscular exercise.

We can hardly see how this is possible in the case of the red corpuscles which remain in a closed, constantly circulating system. Further, if this conclusion is correct, we would expect an increase in the number of erythrocytes in the pig.

Schneider and Havens (7) conclude that the increase in erythrocytes is due to a concentration in the peripheral capillaries. Willebrand (5) believes that the withdrawal of water from the blood by the working muscles is the primary cause of concentration. Zuntz and Schumberg (5, 7) accept Willebrand's explanation.

If either of these conclusions is correct, we would expect the blood of the pig to show an increase in the erythrocytes following exercise. Tornow (2) concluded that the increase in the red corpuscles corresponded roughly to the increased density of the blood as a result of sweat caused by muscular work. Similar conclusions can be drawn from the work of Hasselbalch and Heyerdahl (2), since there was no definite reaction after the first run, while after the second run there was a very distinct rise in the number of red corpuscles. This has been explained by Boothby and Berry (2) on the ground that there was no distinct change in the relative number of red corpuscles until sufficient time had elapsed for an appreciable amount of sweating to have occurred.

Boothby and Berry conclude from their studies that the increase in the percentage of hemoglobin and red corpuscles occurs under conditions of work causing an appreciable amount of perspiration. If no perspiration occurs, there is no such increase.

Evidence in the pig rather tends to confirm this theory, since the pig is an animal which does not sweat and does not show any increase in the number of red corpuscles under various degrees of muscular exercise. The same has been found to occur in the case of the rabbit, another animal which does not sweat.

## LEUCOCYTES AFTER MUSCULAR EXERCISE

An increase in the number of leucocytes occurs in the pig. This increase was slight, compared to the increase found in man.

Schneider and Havens (7) found that the normal proportion of leucocytes usually returned about 30 to 45 minutes after the exercise period. It is quite possible that in the pig a similar phenomena occurred and that the normal proportions had nearly returned by the time we made our after-exercise counts. This fact needs further investigation.

To account for the leucocytosis, Hawk (5) concludes that it is due to an accumulation of leucocytes in the peripheral circulation. Zuntz and Schumberg (5, 7) believe that, since the white corpuscles increase so much more than the red, a different explanation must obtain for their increase. They hold that the passing of wandering cells from the tissues into the general circulation is an adequate explanation. Schneider and Havens conclude that concentration of blood in the peripheral capillaries is the chief cause of the increase in the number of leucocytes. They also state that the contraction of the voluntary muscles accelerates the flow of lymph, throwing lymph rich in leucocytes into the blood.

Our results in the pig show that the influences which caused an increase in the number of red corpuscles in the blood of man can not be used to explain the increase in the number of leucocytes. It would seem that the explanation proposed by other workers—namely, that leucocytosis results from the passage of leucocytes from the tissues and lymphatic system into the general circulation as a result of muscular contraction—is quite satisfactory.

## ADRENALIN THEORY

Schneider and Havens (7) think that adrenalin is primarily responsible for the changes in the number of blood corpuscles in the peripheral circulation. They state that during muscular inaction a large mass of the blood is directed to the splanchnic area, where it probably stagnates and gives up plasma as lymph. There is also throughout the remainder of the body, especially in the limbs, an accumulation of lymph. With the onset of muscular activity the carbon-dioxid content of the blood rises, this carbon dioxid stimulates the central nervous centers which regulate the secretion of the suprarenal glands, hence, the output of adrenalin is increased. The adrenalin causes a constriction of the blood vessels of the splanchnic area; this forces the stagnant red corpuscles into the general circulation, thus giving the rise in specific gravity, hemoglobin, erythrocyte, and leucocyte content of the peripheral blood. The increase in red corpuscles and hemoglobin makes it possible to supply more readily the greater demand for oxygen made by the active muscles. Shortly after the close of the exercise the carbon-dioxid content of the blood falls below normal. As a result the discharge of adrenalin becomes subnormal and the blood once more accumulates in the splanchnic area, so that there is a gradual return to the normal composition and even a temporary subnormal content in red corpuscles.



This is a beautiful explanation and the work of Schneider and Havens tends to prove this theory. If adrenalin is the primary factor concerned in increasing the number of red corpuscles, hemoglobin, etc., we fail to understand why an increase in these factors did not result in the pig.

#### CHANGES IN THE DIFFERENTIAL COUNT

The changes in the percentage of the different kinds of leucocytes can be explained on the theory of rapid aging of the leucocytes due to increased wear. Cells grow old under physiological conditions; it is difficult to follow all of the various stages, because every transition form is not available for presentation, because some of the stages are passed over too quickly, or because certain stages in the life history of different cell types may be very similar to each other. Transitional forms may be met with which can not be named, because nomenclature itself is incomplete.

The signs of old age in a cell are (4, p. 18): (1) The cell body becomes relatively larger; (2) the nucleus becomes spherical and relatively smaller; and (3) the nucleus becomes indented and polymorphous.

A cell with a round single nucleus is younger than a cell with a polymorphous nucleus. In the blood of the pig, following muscular exercise and exposure to the sun's heat, we find a decrease in the mononuclear elements and an increase in the polymorphonuclear elements, showing that the cells are becoming old faster than new cells are being produced or that the rate of aging in a cell has been increased. The mast cells are increased in numbers and the majority of these cells resemble lymphocytes which contain many dark granules. Gruner (4, p. 19) states that the lymphocyte may develop mast-cell granules when suitable conditions arise. Thus, we find a few of the lymphocytes which assume the form of mast cells and may be classed under this heading.

Muscular exercise and increased body temperature both very likely play an important part in hurrying the life cycle in the leucocytes.

#### VARIATIONS IN BODY TEMPERATURE

It is quite evident that the heat-regulating mechanism in the hog is a very poor one. Schroeder and Mohler (8) call attention to the fact that the hog is an animal that is ordinarily incased in a thick layer of fat, which is a poor conductor of heat and in which the circulation of blood is very meager. Over the fat a skin is stretched in which the circulation of blood is relatively small; and this skin, unlike that of a man or a horse, does not take a prominent part in regulating the body temperature through the agency of radiation and perspiration. The covering of a hog may be regarded as an excellent means for preventing the escape of heat from the body rather than for regulating the temperature of the body; hence, we have conditions that probably permit a more rapid production than escape of heat. These workers also state that when the temperature of a number of hogs is compared the difference found is of



such magnitude that they were at a loss to conclude what should be regarded as normal.

There are several factors which can possibly account for the wide variations found in the temperature of normal hogs: (1) The condition of the animal—that is, the amount of fat; (2) the temperature of the atmosphere and the percentage of humidity. A fat hog would have a much higher body temperature on a hot humid day than a lean hog, and even on a cool day a slight variation may occur. Schroeder and Mohler conclude that fat hogs have a higher temperature than lean ones, and that a higher temperature induced by exercise or some other temporary cause persists longer in fat than lean hogs.

Since environment can cause a marked variation in the temperature of man, with his excellent heat-regulating mechanism, we would expect that similar conditions would cause a greater variation in the case of the pig with a poor heat-regulating mechanism.

#### SUMMARY

(1) Blood examinations in normal resting pigs, covering a period of 24 hours, may be quite uniform; but in some animals there is marked variation throughout the period.

(2) Observations made upon a number of animals leads to the conclusion that muscular exercise does not cause an increase of red corpuscles in the peripheral circulation of the pig.

(3) Results based on only one or a few experiments may lead to wrong conclusions, owing to the variability in the blood of pigs.

(4) Evidence given by work with the pig tends to confirm the theory of perspiration being responsible for the increase in the number of red corpuscles following muscular exercise in man.

(5) Muscular exercise in the pig is usually followed by a leucocytosis.

(6) This leucocytosis is probably the result of muscular exercise forcing leucocytes into the general circulation from the tissues.

(7) Muscular exercise leads to marked changes in the differential counts. The mononuclear elements are decreased, and the polymorphonuclear elements are increased. The height of the curve is reached several hours after exercise, and the normal proportions do not return for many hours.

(8) Exposure to the sun causes similar changes in the differential curve.

(9) These changes under both conditions are the result of increased rate of aging of the leucocytes, the cells becoming older faster than young cells are being produced.

(10) Muscular exercise and heat of the sun lead to a marked increase in body temperature.

(11) Body temperature changes are more pronounced in fat pigs than lean ones, but even in pigs weighing 75 to 100 pounds marked changes are likely to occur.

(12) Increased atmospheric temperature and increased percentage of humidity lead to increased body temperature.

(13) Blood examinations of pigs which are to be used for clinical records should be taken from animals which have been confined in a small cool pen for at least 24 hours, and better, 48 hours. The animals must be kept absolutely quiet and not worried. Feeding and watering should be regular. The daily blood examinations should be made at the same time on each day.

(14) Temperature records which are to be used for clinical records should be taken from pigs kept in a cool, shady pen. The animals should not be exercised or worried when the temperatures are taken. If the animals are chased around the pen in endeavoring to obtain the temperature, the last temperatures taken may show a marked rise. For tuberculin work where the temperatures are used it would be best to keep them confined in a crate throughout the test.

(15) The condition of the animal (amount of fat), the temperature of the atmosphere, and the percentage of humidity are factors which should be considered in determining the normal temperature of the pig.

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## RELATION OF THE TRANSFORMATION AND DISTRIBUTION OF SOIL NITROGEN TO THE NUTRITION OF CITRUS PLANTS<sup>1</sup>

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### INTRODUCTION

The total nitrogen content of Citrus lands is frequently low (1)<sup>2</sup>, and it is well recognized that the quantity of available nitrogen formed through the natural processes of nitrification soon becomes inadequate for the needs of Citrus plants unless an effort is made to maintain the nitrogen supply by the addition of commercial fertilizers, cover crops, manure, etc. It has been suggested (7) that the scarcity of organic nitrogen in semiarid soils is offset, to some extent at least, by the great depth to which nitrification takes place in these soils; but, while it is true that nitrification may take place at greater depth in semiarid soils than in humid soils, there seems to be little doubt that the bulk of the nitrates formed in semiarid soils are formed in the surface layers. Few soils are plowed deeper than 9 inches; consequently the cover crops, fertilizers, manure, or other nitrogenous material added are confined to this layer, and the nitrification process naturally is most vigorous in the soil to which nitrogenous organic matter is added from time to time.

In Citrus groves the common practice is to cultivate frequently to a depth of about 6 inches. Comparatively few feeding roots can therefore exist in the upper 6 inches of soil. If the rainfall in southern California was sufficient and was evenly distributed throughout the year, the nitrates formed or carried above the feeding roots by capillarity would be moved downward and brought within reach of the roots at frequent intervals. But the rainfall between April and December is usually too scanty to cause a movement of nitrates from the surface layers of soil; and, as the furrow system of irrigation, which is used most

<sup>1</sup> The work discussed in this paper was carried out in cooperation with the University of California Citrus Experiment Station and Graduate School of Tropical Agriculture at Riverside. The writer wishes to express his indebtedness to Director H. J. Webber and members of his staff for many courtesies and facilities extended during the course of the work.

<sup>2</sup> Reference is made by number to "Literature cited," p. 251-252.

extensively, can not be depended upon to cause a downward movement of the soluble salts from the surface soil, the problem of securing a proper distribution of nitric nitrogen in Citrus soils at once suggests itself.

In many soils a dense plowsole is formed during the irrigation season. The plowsole retards the downward movement of the water, which, under the furrow system, is applied in small streams, several feet apart and several inches below the surface. As the downward movement of the irrigation water is interrupted until the plowsole is softened, which frequently requires several hours, the water moves laterally and upward, not only causing a very uneven distribution of nitrates, but carrying them farther away from the feeding roots. It is therefore obvious that the satisfactory solution of the nitrogen problem in semiarid soils, and especially in furrow-irrigated soils, not only depends upon a knowledge of the factors influencing nitrification, but upon the forces controlling the distribution of nitric nitrogen, which is presumably the most valuable source of nitrogen for crops.

#### ARRANGEMENT OF EXPERIMENT PLOTS

The major portion of the work presented in this paper has been carried out on the soils of the Citrus Experiment Station grove, at Riverside, Cal.; and it therefore seems desirable to include a brief description of the field and also a diagram (fig. 1) showing the arrangement of plots and the fertilizer applied.

The experimental field was laid out from virgin land in April, 1907. The arrangement of the field is such that each plot is surrounded on all sides by a guard row which effectively prevents the treatment on one plot from influencing the trees on any other. The irrigation of each plot is separate, and no waste water or tailings from one plot is allowed to pass to any other part of the grove.

In planning the experiment a uniform system of fertilization was adopted and each plot has been given the same kind of fertilizer each year, the quantity applied increasing in amount with the development of the trees. During the last four years plots A, C, G, H, L, Q, and S have received each year 1.35 pounds of nitrogen per tree. As there are 108 trees per acre, the applied nitrogen amounts to 145.8 pounds for each acre of land. Plots F and O have received approximately the same quantity of nitrogen in manure until the last year, when the amount applied was in excess of the amount applied to the above-mentioned plots. Plots U and V, which were not originally included in the fertilizer experiment, have received moderate applications of manure in addition to a cover crop of vetch each year. Plots E, K, N, and P have received each year during the last four years about 0.45 pound of nitrogen per tree, or about 48.6 pounds of nitrogen per acre. Plots B, D, I, J, M, R, and T have received no nitrogenous fertilizers at any time.

Road		Road		Road		Road	
<b>Plot U</b> Vetch cover crop, barn- yard manure: Pounds. 1914-15..... 175 1916..... 280	<b>Plot A</b> Pounds. Nitrate of soda..... 4 Blood..... 2 Potassium sulphate.. 3 Bone..... 14	<b>Plot F</b> Pounds. Manure: 1914-15..... 227 1916..... 350	<b>Plot K</b> Pounds. Bone..... 14 Potassium sulphate.. 3	<b>Plot P</b> Pounds. Bone..... 14			
	<b>Plot B</b> No fertilizer.	<b>Plot G</b> Pounds. Nitrate of soda..... 4 Blood..... 4 Bone..... 14	<b>Plot L</b> Pounds. Nitrate of soda..... 5 Blood..... 3 Potassium sulphate... 3	<b>Plot Q</b> Pounds. Nitrate of soda..... 4 Blood..... 5 Superphosphate..... 13 Potassium sulphate... 3			
	<b>Plot C</b> Pounds. Blood..... 10	<b>Plot H</b> Pounds. Nitrate of soda..... 9	<b>Plot M</b> No fertilizer.	<b>Plot R</b> Pounds. Potassium sulphate.. 3			
	<b>Plot D</b> Pounds. Potassium sulphate.. 3	<b>Plot I</b> Pounds. Muriate of potash... 3	<b>Plot N</b> Pounds. Blood..... 2.5 Superphosphate.... 13.0	<b>Plot S</b> Pounds. Blood..... 10			
	<b>Plot E</b> Pounds. Bone..... 14	<b>Plot J</b> Pounds. Superphosphate..... 13	<b>Plot O</b> Pounds. Manure: 1914-15..... 227 1916..... 350 Rock phosphate.... 6	<b>Plot T</b> No fertilizer.			
						<b>Plot V</b> Vetch cover crop, barn- yard manure: Pounds. 1914-15..... 175 1916..... 280	

Fig. 1.—Diagram of Citrus Experiment Station grove, Riverside, Cal., showing the arrangement of the plots and the kind and quantity of fertilizers applied.

FIG. 1.—Diagram of Citrus Experiment Station grove, Riverside, Cal., showing the arrangement of the plots and the kind and quantity of fertilizers applied.



## CHANGES IN THE NITROGEN CONTENT OF SOILS FOLLOWING THE ADDITION OF BLOOD OR OTHER ORGANIC SUBSTANCES<sup>1</sup>

In the studies on the changes in the nitrogen content of soils following the addition of nitrogenous materials, a representative sample of soil was secured from each field or plot to be studied by making a large number of borings to a depth of 12 inches. The soil was then thoroughly mixed and passed through a clean sieve to remove gravel, roots, etc., and held in a closed container until the moisture content could be determined. A sufficient quantity of the soil was then weighed out to make 1 kgm. of dry soil, the desired quantity of the substance to be studied was added, thoroughly mixed with the soil, and the moisture content brought up to the optimum. The incubation was carried out in 1-quart Mason jars held at a temperature of 28° C., the optimum moisture content being maintained at all times. When green plants or other substances were added, they were first passed through a grinder which rendered them sufficiently fine to make it possible to secure a uniform distribution in the soil. With the exception of dried blood, the moisture was determined in all materials used, and the addition based on the dry weight.

The ammonia was determined by extracting the soil with 10 per cent hydrochloric acid, rendering an aliquot part of the filtrate alkaline with sodium hydroxid, and then distilling. The reason for using a 10 per cent acid will be discussed at length in a second paper, which will deal especially with the determination of ammonia in semiarid soils. The nitrates were determined by the aluminium-reduction method, which gave very satisfactory results when the reduction was allowed to take place for 24 hours, and an acid trap used to prevent any loss of nitrogen during the reduction period. All determinations were made in duplicate, and, in case there was not a close agreement, additional analyses were made.

The rate at which ammonia and nitrates are formed from green plant material is controlled in a large measure by the maturity of the plants, and in order to secure a fair comparison of different plants it is necessary that they be taken at the same stage of maturity. All plants used in the following experiment were therefore selected at the flowering stage, unless otherwise stated in the text.

The total nitrogen determinations were all made in triplicate by the Kjeldahl-Gunning-Jodblauer process.

On April 8, 1914, soil samples were taken from three plots in the Citrus Experiment Station grove and the transformation of nitrogen, following the addition of blood or other nitrifiable materials, studied. The ammonia and nitrates were determined at 7-day intervals for a period of 6 weeks. At the end of the incubation period the total nitrogen was determined, and the gain or loss of nitrogen during the incubation period calculated from the original nitrogen content of the soil and the nitrogen added in the dried blood or plant substances.

<sup>1</sup> In all ammonification or nitrification studies in which dried blood or other nitrogenous materials were used, the figures given represent the gain in ammonia or nitrates over the amount found in the control samples, which received water only.

TABLE I.—*Nitrification of dried blood, barley hay, horse manure, and green manures in Citrus Experiment Station soils, Riverside, Cal. April 8, 1914*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

## PLOT E

Material added.	Constituent.	Incubation period.					
		7 days.	14 days.	21 days.	28 days.	35 days.	42 days.
Dried blood (1 per cent) ..	Ammonia..	35.28	33.20	25.66	25.48	25.76	25.48
Do.....	Nitrates...	3.04	30.94	47.31	46.37	49.61	47.29
Oats (1 per cent).....	Ammonia..	.56	1.28	1.12	.28	.46	.84
Do.....	Nitrates...	.09	.57	.74	.46	.38	.33
Barley (1 per cent).....	Ammonia..	.56	.16	— .09	— .28	.00	.21
Do.....	Nitrates...	.51	.42	.53	— .03	2.57	1.38
Melilotus (1 per cent)....	Ammonia..	2.24	.72	.56	1.40	1.68	1.40
Do.....	Nitrates...	3.65	5.23	8.70	11.40	10.22	8.73
Alfilaria (1 per cent) ....	Ammonia..	1.12	.16	.14	.28	.68	.28
Do.....	Nitrates...	.73	.84	.98	1.28	1.32	2.02
Water only .....	Ammonia..	1.12	.96	1.12	1.40	1.12	.84
Do.....	Nitrates...	.96	1.40	1.68	2.40	2.24	2.80

## PLOT D

Dried blood (1 per cent) ..	Ammonia..	47.92	54.24	58.44	46.36	47.96	45.02
Do.....	Nitrates...	.99	— 1.40	— .99	6.27	13.69	15.75
Oats (1 per cent).....	Ammonia..	.00	.56	.00	.44	.00	.28
Do.....	Nitrates...	.01	— 1.77	— .01	1.21	1.57	2.83
Barley (1 per cent).....	Ammonia..	.00	1.12	.56	.58	.56	.44
Do.....	Nitrates...	1.48	— 1.77	— .77	2.81	3.45	3.83
Melilotus (1 per cent)....	Ammonia..	1.68	1.68	.00	.00	.00	.00
Do.....	Nitrates...	2.07	2.58	3.06	5.90	6.01	7.77
Alfilaria (1 per cent) ....	Ammonia..	.56	.56	.00	.44	.56	.40
Do.....	Nitrates...	1.81	.33	1.01	2.85	2.87	3.45
Water only .....	Ammonia..	1.68	1.12	1.12	1.24	1.12	.96
Do.....	Nitrates...	1.25	3.54	3.09	1.95	2.22	2.18

## PLOT C

Dried blood (1 per cent) ..	Ammonia..	33.44	34.72	31.36	24.64	25.20	24.08
Do.....	Nitrates...	3.10	7.39	15.31	17.46	28.99	29.41
Barley hay (1 per cent) ..	Ammonia..	— .16	— .44	.00	.00	.00	.00
Do.....	Nitrates...	— 1.31	— 1.18	— .66	— .72	— .63	— .42
Barley (1 per cent).....	Ammonia..	.40	.80	.00	.56	.55	.00
Do.....	Nitrates...	1.56	3.73	3.98	3.28	2.89	2.37
Melilotus (1 per cent) ...	Ammonia..	1.96	.56	.00	— .42	.00	.00
Do.....	Nitrates...	2.62	3.90	3.76	7.29	7.39	8.37
Horse manure (1 per cent)	Ammonia..	.00	— .56	.00	.00	.00	.00
Do.....	Nitrates...	— .42	.45	— .66	.86	1.07	1.29
Water only .....	Ammonia..	1.84	1.68	1.68	1.68	1.12	1.12
Do.....	Nitrates...	1.73	1.70	2.65	2.88	2.36	2.83

In studying the figures presented in Table I it is seen that the increase in ammonia resulting from the application of 1 per cent of blood reaches the maximum in soil E after 7 days, in soil C after 14 days, and in soil D not until after 21 days. It is observed that the ammonia is uniformly higher in soil D than in soil E or C. Some increase in nitrates is observed

in all of the soils after the first 7 days, and in soil C the amount increases somewhat uniformly until the end of the experiment. In soil E a rapid increase in nitrates is noted from 7 to 21 days; but during the last 3 weeks the gain is irregular and very slow. In soil D a loss of nitrates occurs between 7 to 21 days. It is interesting to note that during this period the ammonia is very high, and there is a possibility of the action of the nitrifying organism being inhibited by the presence of so much ammonia.

By referring to Table II it is seen that the percentage of nitrogen recovered as ammonia varies from 17.55 in soil C to 32.81 in soil D, while the percentage of the nitrogen recovered as nitrates varies from 11.48 in soil D to 34.47 in soil E. The average loss of nitrogen from the three soils following the addition of 1 per cent of blood is 29.25 per cent of the nitrogen added.

TABLE II.—Percentage of nitrogen added to soils in dried blood, barley hay, horse manure, and green manures recovered as ammonia and nitrates after six weeks' incubation; also percentage gain or loss of nitrogen added. April 8, 1914

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

PLOT E								
Material added	Nitrogen in material added.	Nitrogen as ammonia recovered in 6 weeks.	Percentage of nitrogen as ammonia recovered.	Nitrogen as nitrate recovered in 6 weeks.	Percentage of nitrogen as nitrate recovered.	Nitrogen remaining in soil after 6 weeks.	Gain or loss.	Percentage of nitrogen gained or lost.
Dried blood (1 per cent).....	137.20	25.48	18.57	47.29	34.47	92.80	-44.40	-32.36
Oats, green (1 per cent).....	16.90	.84	4.97	.33	1.95	33.00	16.10	95.27
Barley, green (1 per cent).....	14.20	.21	1.48	1.38	9.72	24.50	10.30	72.53
Melilotus, green (1 per cent)....	21.20	1.40	6.60	8.73	41.18	34.00	12.80	60.38
Alfilaria, green (1 per cent)....	18.80	.28	1.49	2.02	10.74	26.60	7.80	41.49
PLOT D								
Dried blood (1 per cent).....	137.20	45.02	32.81	15.75	11.48	95.20	42.00	-30.61
Oats, green (1 per cent).....	16.90	.28	1.66	2.83	16.74	29.20	12.30	72.78
Barley, green (1 per cent).....	14.20	.44	3.10	3.83	26.97	28.10	13.90	97.89
Melilotus, green (1 per cent)....	21.20	.00	.00	7.77	36.65	35.30	14.10	66.51
Alfilaria, green (1 per cent)....	18.80	.40	2.13	3.45	18.35	28.00	9.20	48.94
PLOT C								
Dried blood (1 per cent).....	137.20	24.08	17.55	29.41	21.44	103.20	-34.00	-24.78
Barley hay (1 per cent).....	13.60	.00	.00	-.42	-3.09	16.10	2.50	18.38
Barley, green (1 per cent).....	14.20	.00	.00	2.37	16.69	19.60	5.40	38.03
Melilotus, green (1 per cent)....	21.20	.00	.00	8.39	39.58	31.40	10.20	48.11
Horse manure (1 per cent).....	13.60	.00	.00	1.29	9.49	21.00	7.40	54.41

The addition of 1 per cent of oats caused a moderate increase in ammonia in soil E, but very little increase in soil D. However, after 42 days' incubation the ammonia content of both soils was somewhat higher than in the controls. It would seem that very little increase in nitrates resulted from the addition of the oats to soil E, and the increase in soil D amounts to only 2.83 mgm. The percentage of nitrogen recovered as ammonia was 4.97 in soil E and 1.66 in soil D. The percentage of

nitrogen recovered as nitrates in soil E was only 1.95, but in soil D the percentage recovered as nitrates amounted to 16.74. Both of the soils to which oats were added show gains in total nitrogen, amounting to 95.27 per cent of the nitrogen added in soil E and 72.78 per cent in soil D.

The addition of 1 per cent of green barley caused only slight increases in ammonia, and the increase in nitrates after 6 weeks' incubation varied from 1.38 mgm. in soil E to 3.83 mgm. in soil D.

One per cent of melilotus caused a decided gain in ammonia in all soils during the early part of the incubation period, and in soil E the increase in ammonia caused by the decay of the melilotus is apparent throughout the experiment, but after 21 days soils D and C contained no more ammonia than the controls.

The increase in nitric nitrogen resulting from the addition of green melilotus begins during the first few days. After seven days' incubation the average gain for the three soils is more than the average gain from oats or barley during the entire six weeks' incubation. In soil E the maximum increase in nitrates is obtained after 28 days. In soils D and C the gain continues throughout the six weeks; but the total increase is less than in the soil in which the maximum gain is attained in a shorter period.

None of the nitrogen added in melilotus in soils C and D remained in the soil as ammonia, but 6.6 per cent remained in soil E. Nearly 40 per cent of the nitrogen added in melilotus was recovered as nitrates and the nitrogen gains in the three soils varied from 48.11 per cent of the nitrogen added in soil C to 66.51 per cent in soil D.

The addition of 1 per cent of alfalfa showed an increase in ammonia after seven days, but after the seven-day period the increase over the controls was very small. Small gains in nitrates were secured in seven days, and the increase proceeded gradually during the six weeks' incubation.

A little less than 2 per cent of the nitrogen added in alfalfa was recovered as ammonia, and from 10.74 to 18.35 per cent as nitrates. The nitrogen gain from alfalfa is smaller than from oats, barley, or melilotus.

The addition of barley hay showed no increase in ammonia at any time during the experiment. Neither was there any increase in nitrates in this soil over the supply in the control, thus indicating that the nitrification of mature barley is much slower than the nitrification of green manures.

Horse manure was used only in soil C, in which it caused no apparent increase in ammonia over the control. Nitrification proceeded slowly and somewhat irregularly. The gain in nitrates over the control amounts to only 1.29 mgm. in six weeks. Only 9.49 per cent of the nitrogen added in horse manure was recovered as nitrates. However, there was a gain in nitrogen amounting to 54.41 per cent of the nitrogen added.



The ammonia content of the control soils remained rather constant throughout the experiment, but the nitric nitrogen increased slowly and somewhat irregularly. At the conclusion of the experiment, the average nitrate content in the three soils amounts to 2.61 mgm. per 100 gm. of soil, which would be approximately 100 pounds of nitrogen per acre-foot of soil. This rate of nitrification would seem to be sufficient to supply the needs of any crop. However, if these soils had remained in the field undisturbed except by the ordinary cultivation, it is doubtful whether the increase in nitrates would have amounted to more than a fraction of the gains secured under laboratory conditions.

On June 23, 1914, three soil samples were secured from Highland, Cal. One sample was taken from a productive grove, one from an unproductive grove, and a third from an adjacent virgin soil. The nitrogen changes in these soils following the addition of blood, red clover, or alfalfa are shown in Tables III and IV.

TABLE III.—*Nitrification of dried blood and green manures in soils from Highland, Cal. June 23, 1914*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

PRODUCTIVE SOIL							
Material added.	Constituent.	Incubation period.					
		7 days.	14 days.	21 days.	28 days.	35 days.	42 days.
Dried blood (1 per cent).	Ammonia..	29.12	38.36	33.04	36.40	32.76	32.20
Red clover (1 per cent.)..	Nitrates...	.56	2.80	10.08	9.84	8.96	8.96
Do.....	Ammonia..	— .00	— .56	.28	— .28	.28	.00
Do.....	Nitrates...	.84	3.48	5.90	7.64	9.52	11.48
Alfalfa (1 per cent.).....	Ammonia..	1.68	— .28	1.12	.00	.56	.56
Do.....	Nitrates...	3.92	10.08	13.44	14.84	16.24	15.96
Water only.....	Ammonia..	1.68	1.96	1.12	1.40	1.12	1.12
Do.....	Nitrates...	1.40	1.40	2.24	1.96	2.24	1.96
UNPRODUCTIVE SOIL							
Dried blood (1 per cent.)..	Ammonia..	32.20	38.40	38.08	34.88	34.16	33.88
Do.....	Nitrates...	.28	.28	4.20	5.00	7.00	8.96
Red clover (1 per cent.)..	Ammonia..	— .28	— .28	.00	.24	.00	.00
Do.....	Nitrates...	1.12	2.25	2.24	3.80	5.60	7.00
Alfalfa (1 per cent.).....	Ammonia..	4.20	2.24	.28	.74	.84	.84
Do.....	Nitrates...	7.28	15.40	14.84	17.26	18.20	18.76
Water only.....	Ammonia..	1.68	1.40	1.40	1.12	1.40	1.12
Do.....	Nitrates...	1.12	1.40	1.68	1.80	1.96	1.68
VIRGIN SOIL							
Dried blood (1 per cent.)..	Ammonia..	21.28	26.32	20.40	37.86	29.48	28.00
Do.....	Nitrates...	1.12	8.68	20.20	18.76	19.92	19.04
Red clover (1 per cent.)..	Ammonia..	— .28	.28	.24	.42	.14	.28
Do.....	Nitrates...	.28	1.68	3.08	7.28	7.96	11.48
Alfalfa (1 per cent.).....	Ammonia..	— .28	.28	.28	.14	.28	.00
Do.....	Nitrates...	.84	3.92	8.40	10.08	12.52	13.72
Water only.....	Ammonia..	1.40	1.12	1.12	.98	1.12	1.12
Do.....	Nitrates...	1.12	1.40	1.68	1.40	1.68	1.40



TABLE IV.—Percentage of nitrogen added to Highland, Cal., soils in dried blood and green manures recovered as ammonia and nitrates after six weeks' incubation; also percentage gain or loss of nitrogen added. June 23, 1914

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

PRODUCTIVE SOIL								
Material added.	Nitrogen in material added.	Nitrogen as ammonia recovered in 6 weeks.	Percentage of nitrogen as ammonia recovered.	Nitrogen as nitrate recovered in 6 weeks.	Percentage of nitrogen as nitrate recovered.	Nitrogen remaining in soil after 6 weeks.	Gain or loss in nitrogen.	Percentage of of nitrogen gained or lost.
Dried blood (1 per cent).....	137.20	32.00	23.32	8.96	6.53	62.90	-74.30	-54.15
Red clover (1 per cent).....	20.07	.00	.00	11.48	57.20	30.70	10.63	52.96
Alfalfa (1 per cent).....	28.60	.56	1.96	15.96	55.81	39.10	10.50	36.71
UNPRODUCTIVE SOIL								
Dried blood (1 per cent).....	137.20	33.88	24.69	8.96	6.53	67.50	-69.70	-50.80
Red clover (1 per cent).....	20.07	.00	.00	7.00	34.88	26.90	6.83	34.03
Alfalfa (1 per cent).....	28.60	.84	2.94	18.76	65.59	38.10	9.50	33.20
VIRGIN SOIL								
Dried blood (1 per cent).....	137.20	28.00	20.41	19.04	13.88	66.00	-71.20	-51.89
Red clover (1 per cent).....	20.07	.28	1.37	11.48	57.20	25.00	4.93	24.56
Alfalfa (1 per cent).....	28.60	.00	.00	13.72	47.97	35.00	6.40	22.38

The addition of 1 per cent of dried blood to these soils caused a rapid increase in the ammonia content. At the end of the first seven days the ammonia in these three soils varied from 21.28 to 32.2 mgm. per 100 gm. of soil, and there was little tendency for the ammonia to decrease during the latter part of the incubation period. The percentage of nitrogen recovered as ammonia, as shown in Table IV, varied from 20.41 in the virgin soil to 24.69 in the unproductive soil. The increase in nitric nitrogen following the addition of 1 per cent of dried blood was rather low in these soils. In the productive soil and also in the virgin soil the maximum increase in nitrates was secured after 21 days. In the unproductive soil there was a slow but steady increase in nitrates throughout the incubation period. The percentage of nitrogen recovered as nitrates is in all cases less than the percentage recovered as ammonia. The loss of nitrogen was very heavy in all of these soils, the average for the three soils being above 50 per cent of the nitrogen added.

When 1 per cent of red clover was added, there was a reduction in the ammonia content of the virgin and unproductive soils during the first seven days and no increase in the productive soil. At no time during the incubation period of six weeks did the increase in ammonia amount to more than 0.42 mgm. per 100 gm. of soil. At the conclusion of the experiment, none of the nitrogen added in red clover was recovered as ammonia in the productive or unproductive soils and only 1.37 per cent in the virgin soil. The increase in nitric nitrogen from the addition of

red clover began during the first seven days and continued to increase rather uniformly throughout the experiment. At the conclusion of the experiment, from 34.88 to 57.2 per cent of the nitrogen added was recovered as nitrates. The increase in total nitrogen in the soils receiving red clover varied from 24.56 per cent in the virgin soil to 52.96 per cent in the productive soil.

The addition of 1 per cent of alfalfa caused considerable increase in the ammonia content of the productive and unproductive soils during the first seven days, but a slight reduction in the virgin soil. During the latter part of the incubation period the increase in ammonia in these soils is very small, and the percentage of nitrogen recovered as ammonia at the conclusion of the experiment varied from nothing in the virgin soil to 2.94 in the unproductive soil. Considerable increase in nitric nitrogen took place during the first seven days in the productive and unproductive soils, but very little increase in the virgin soil. The nitric nitrogen continued to increase throughout the incubation period, and at the end of the six weeks the percentage of nitrogen recovered as nitrates from alfalfa varied from 47.97 in the virgin soil to 65.59 in the unproductive soil. There was a gain in total nitrogen in each of the soils to which alfalfa was added.

When these soils were incubated without the addition of nitrogenous materials, the ammonia content remained quite uniform throughout the incubation period; but there was an appreciable increase in nitrates, which seemed to have reached a maximum after 21 days in the productive and virgin soil and after 35 days in the unproductive soil.

On July 16, soil samples were secured from four plots in the experimental field at the Citrus Experiment Station, Riverside, Cal., and one sample from an adjacent virgin soil. Each of these soils was divided into four portions of 1 kgm. each, one portion from each soil receiving 1 per cent of dried blood. Each of the other portions received 2 per cent of red clover, alfalfa, or buckwheat. The results obtained in this experiment are presented in Table V.

When 1 per cent of dried blood was added to these soils, there was a considerable increase in the ammonia content of each of the five soils included in the experiment. The percentage of nitrogen recovered as ammonia at the end of six weeks varied from 18.25 in soil U to 38.37 in the virgin soil. The increase in nitric nitrogen in these soils is extremely variable. In the virgin soil and soil B only 1.22 per cent of the nitrogen added in dried blood was recovered as nitrates, while in soils F and U 32.65 and 36 per cent, respectively, were recovered. The percentage of nitrogen lost varied from 13.05 in plot F to 47.96 in plot B.

When 2 per cent of red clover was added, there was an appreciable increase in the ammonia content of all of the soils. However, the average percentage of nitrogen recovered as ammonia is only 4.5. The lowest increase in nitrates is in the virgin soil and amounts to 26.51 per cent

of the nitrogen added. With the exception of soil B, there is an increase in total nitrogen following the addition of the clover, which in soil U amounts to 32.54 per cent of the nitrogen added.

TABLE V.—Percentage of nitrogen added to soils in dried blood and green manures recovered as ammonia and nitrates after six weeks' incubation; also percentage gain or loss of nitrogen added. July 16, 1914

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

PLOT U								
Materials added.	Nitrogen in material added.	Nitrogen as ammonia recovered in 6 weeks.	Percentage of nitrogen as ammonia recovered.	Nitrogen as nitrate recovered in 6 weeks.	Percentage of nitrogen as nitrate recovered.	Nitrogen remaining in soil after 6 weeks.	Gain or loss in nitrogen.	Percentage of nitrogen gained or lost.
Dried blood (1 per cent).....	137.20	25.04	18.25	49.40	36.00	108.60	-28.60	-20.85
Red clover (2 per cent).....	40.14	1.96	4.88	14.56	36.27	53.20	13.06	32.54
Alfalfa (2 per cent).....	57.20	2.24	3.92	17.64	30.84	75.60	18.40	32.17
Buckwheat (2 per cent).....	33.12	1.40	4.23	8.12	24.52	35.00	1.88	5.68
PLOT F								
Dried blood (1 per cent).....	137.20	33.04	24.08	44.80	32.65	119.30	-17.90	-13.05
Red clover (2 per cent).....	40.14	1.40	3.49	16.24	40.46	50.69	10.55	26.28
Alfalfa (2 per cent).....	57.20	1.68	2.94	19.04	33.29	70.50	13.30	23.25
Buckwheat (2 per cent).....	33.12	.84	2.54	2.52	7.61	33.90	.78	2.36
PLOT B								
Dried blood (1 per cent).....	137.20	50.68	36.94	1.68	1.22	71.40	-65.80	-47.96
Red clover (2 per cent).....	40.14	2.24	5.59	12.04	30.00	40.14	.00	.00
Alfalfa (2 per cent).....	57.20	2.24	3.92	14.00	24.48	51.80	-5.40	-9.44
Buckwheat (2 per cent).....	33.12	1.68	5.07	1.12	3.38	25.22	-7.90	-23.85
PLOT H								
Dried blood (1 per cent).....	137.20	51.40	37.46	5.76	4.20	98.70	-38.50	-26.06
Red clover (2 per cent).....	40.14	1.68	4.19	12.88	32.09	44.10	3.96	9.87
Alfalfa (2 per cent).....	57.20	2.24	3.92	15.12	26.43	53.90	-3.30	-5.77
Buckwheat (2 per cent).....	33.12	1.12	3.38	1.40	4.23	23.10	-10.02	-30.25
VIRGIN SOIL								
Dried blood (1 per cent).....	137.20	52.64	38.37	1.68	1.22	81.20	-56.00	-40.80
Red clover (2 per cent).....	40.14	1.68	4.19	10.64	26.51	49.00	8.86	22.07
Alfalfa (2 per cent).....	57.20	1.68	2.94	14.56	25.46	61.60	4.40	7.69
Buckwheat (2 per cent).....	33.12	1.12	3.38	1.12	3.38	32.20	-.92	-2.78

The addition of 2 per cent of alfalfa caused only a small increase in the ammonia content of the soil, but a marked gain in nitric nitrogen, from 24.48 to 33.29 per cent of the nitrogen in the alfalfa being converted into nitrates. Considerable gains in total nitrogen were secured in soils U and F and a small gain in the virgin soil, but soils B and H showed a small loss of nitrogen.

Buckwheat caused a somewhat smaller increase in the ammonia content of the soil than did red clover or alfalfa. The percentage of nitrogen recovered from buckwheat as nitrates is very much smaller than the

percentage recovered from red clover or alfalfa. Small nitrogen gains were secured from buckwheat in soils U and F, but in soils B and H there was considerable nitrogen lost and a small loss in the virgin soil.

On August 28 a third set of samples was taken from the experimental plots at Riverside and one sample from the adjacent virgin soil. Each sample was divided into nine portions. One portion of each soil was conducted as a control. The other portions received, respectively, 1 per cent of dried blood, 2 per cent of vetch, melilotus, soybeans, corn, cowpeas, black-eyed peas, or oats. The results obtained in this experiment are presented in Tables VI and VII.

TABLE VI.—*Nitrification of dried blood and green manures in Citrus Experiment Station soils, Riverside, Cal. August 28, 1914*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Material added.	Constituent.	Plot U.			Plot H.			Plot B.		
		Incubation period.			Incubation period.			Incubation period.		
		14 days.	28 days.	42 days.	14 days.	28 days.	42 days.	14 days.	28 days.	42 days.
Dried blood (1 per cent)...	Ammonia...	4.34	6.72	6.44	34.44	43.40	32.48	35.56	45.92	47.74
Do.....	Nitrates...	42.28	66.36	70.56	.56	.34	.00	.28	3.92	— .42
Vetch (2 per cent).....	Ammonia...	4.34	1.68	1.68	10.92	9.52	8.12	8.68	17.92	13.58
Do.....	Nitrates...	17.08	24.92	26.32	5.60	12.10	11.48	.56	6.02	14.42
Melilotus (2 per cent).....	Ammonia...	1.82	1.96	1.40	8.96	4.76	.70	9.80	4.76	1.70
Do.....	Nitrates...	14.84	30.24	34.16	5.04	12.99	13.72	2.80	15.12	15.60
Soybeans (2 per cent).....	Ammonia...	.14	.00	— .14	.00	.00	.00	.00	1.68	.26
Do.....	Nitrates...	2.12	7.28	12.04	2.80	9.02	10.64	1.68	7.84	11.34
Corn (2 per cent).....	Ammonia...	.14	.00	.00	.00	.28	.00	.28	.84	1.18
Do.....	Nitrates...	.28	9.24	18.20	.28	4.54	14.84	.84	1.96	17.00
Cowpeas (2 per cent).....	Ammonia...	.30	.28	.00	— .14	.00	.00	.14	1.40	.70
Do.....	Nitrates...	— .70	1.12	22.04	— .56	10.98	12.32	.00	6.72	12.48
Black-eyed peas (2 per cent).....	Ammonia...	4.34	1.68	.98	2.80	1.68	.56	5.60	1.68	1.26
Do.....	Nitrates...	— .56	10.36	18.96	— .56	15.18	12.88	.14	2.08	15.54
Oats (2 per cent).....	Ammonia...	.00	.00	— .28	.00	.28	.00	— .42	.28	.26
Do.....	Nitrates...	— .69	— 1.40	— 1.68	— .56	— .34	— 2.16	.28	— .84	— .84
Water only.....	Ammonia...	.98	1.40	1.40	1.12	1.40	1.12	.84	1.12	.98
Do.....	Nitrates...	1.12	1.96	2.26	1.12	1.90	2.80	.56	1.40	1.26

Material added.	Constituent.	Plot F.			Virgin soil.		
		Incubation period.			Incubation period.		
		14 days.	28 days.	42 days.	14 days.	28 days.	42 days.
Dried blood (1 per cent).....	Ammonia...	24.64	28.14	27.16	36.42	51.44	41.28
Do.....	Nitrates...	11.64	44.24	50.68	— .14	— .28	— .56
Vetch (2 per cent).....	Ammonia...	5.88	.72	.39	.30	14.98	7.20
Do.....	Nitrates...	8.56	15.40	19.32	.14	— .14	— .84
Melilotus (2 per cent).....	Ammonia...	.44	1.54	.74	10.50	17.78	11.60
Do.....	Nitrates...	14.12	38.30	40.88	.00	3.64	13.16
Soybeans (2 per cent).....	Ammonia...	— .16	.32	.28	.00	.14	.40
Do.....	Nitrates...	2.34	9.80	13.44	.00	3.12	7.12
Corn (2 per cent).....	Ammonia...	— .14	.70	.70	3.22	1.26	.34
Do.....	Nitrates...	.02	10.92	18.72	.00	8.68	14.84
Cowpeas (2 per cent).....	Ammonia...	— .28	.22	.28	.28	1.82	.54
Do.....	Nitrates...	— .40	6.16	22.68	— .14	.84	10.08
Black-eyed peas (2 per cent).....	Ammonia...	4.52	1.54	1.68	5.18	10.50	3.20
Do.....	Nitrates...	— .12	13.72	19.84	— .14	3.92	12.88
Oats (2 per cent).....	Ammonia...	— .28	.14	— .14	— .14	.14	— .72
Do.....	Nitrates...	— .40	— .84	— 1.40	— .28	— .56	— 1.26
Water only.....	Ammonia...	1.12	1.26	1.12	.98	1.82	1.28
Do.....	Nitrates...	.96	1.68	1.96	.70	1.12	1.68



TABLE VII.—Percentage of nitrogen added to soils in dried blood and green manures recovered as ammonia and nitrates after six weeks' incubation; also percentage gain or loss of nitrogen added. August 28, 1914

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

PLOT U								
Material added.	Nitrogen in material added.	Nitrogen as ammonia recovered in 6 weeks.	Percentage of nitrogen as ammonia recovered.	Nitrogen as nitrate recovered in 6 weeks.	Percentage of nitrogen as nitrate recovered.	Nitrogen remaining in soil after 6 weeks.	Gain or loss in nitrogen	Percentage of nitrogen gained or lost.
Dried blood (1 per cent).....	137.20	6.44	4.69	70.56	51.43	119.00	-18.20	-13.27
Vetch (2 per cent)....	65.20	1.68	2.58	26.32	40.37	77.00	11.80	18.10
Mellilotus (2 per cent)....	48.40	1.40	2.89	34.16	70.58	86.60	38.20	78.93
Soybeans (2 per cent)....	43.40	.14	.32	12.04	27.74	49.00	5.60	1.29
Corn (2 per cent).....	30.02	.00	.00	18.20	60.61	69.30	39.26	130.20
Cowpeas (2 per cent)....	45.00	.00	.00	22.04	48.98	63.70	18.70	41.50
Black-eyed peas (2 per cent).....	42.40	.98	2.31	18.96	44.72	72.10	19.70	70.05
Oats (2 per cent).....	20.20	-.28	-1.39	-1.68	-8.32	21.70	1.50	7.43
PLOT H								
Dried blood (1 per cent).....	137.20	32.48	23.67	0.00	0.00	57.40	-79.80	-58.16
Vetch (2 per cent)....	65.20	8.12	12.45	11.48	17.61	64.40	-.80	-1.23
Mellilotus (2 per cent)....	48.40	.70	1.45	13.72	28.35	79.80	31.40	64.88
Soybeans (2 per cent)....	43.40	.00	.00	10.64	24.52	44.80	1.40	3.23
Corn (2 per cent).....	30.02	.00	.00	14.84	49.43	75.60	45.58	151.90
Cowpeas (2 per cent)....	45.00	.00	.00	12.32	27.38	58.80	13.80	30.66
Black-eyed peas (2 per cent).....	42.40	.56	1.33	12.88	30.38	63.00	20.60	48.58
Oats (2 per cent).....	20.20	.00	.00	-2.16	-10.69	19.60	-.60	-2.97
PLOT B								
Dried blood (1 per cent).....	137.20	47.74	34.79	0.00	0.00	63.00	-74.20	-54.08
Vetch (2 per cent)....	65.20	13.58	20.83	14.42	22.11	71.40	6.20	9.51
Mellilotus (2 per cent)....	48.40	1.70	3.51	15.60	32.23	74.90	26.50	54.75
Soybeans (2 per cent)....	43.40	.26	.60	11.34	26.13	46.20	2.80	6.45
Corn (2 per cent).....	30.02	1.18	3.93	17.00	56.63	72.10	42.08	140.17
Cowpeas (2 per cent)....	45.00	.70	1.50	12.48	27.73	51.80	6.80	15.11
Black-eyed peas (2 per cent).....	42.40	1.26	2.96	15.54	36.65	50.40	8.00	18.87
Oats (2 per cent).....	20.20	.26	1.29	-.84	-4.16	16.80	-3.40	-16.83
PLOT F								
Dried blood (1 per cent).....	137.20	27.16	19.79	50.68	36.94	112.00	-25.20	-18.37
Vetch (2 per cent)....	65.20	10.39	.60	19.32	29.63	74.20	9.00	13.80
Mellilotus (2 per cent)....	48.40	.74	1.53	40.88	84.46	99.40	51.00	105.37
Soybeans (2 per cent)....	43.40	.28	.65	13.44	30.97	51.80	8.40	19.36
Corn (2 per cent).....	30.02	.70	2.33	18.72	62.36	70.70	40.68	135.51
Cowpeas (2 per cent)....	45.00	.28	.62	22.68	50.40	65.80	20.80	46.22
Black-eyed peas (2 per cent).....	42.40	1.68	3.96	19.84	46.79	76.30	33.90	79.95
Oats (2 per cent).....	20.20	-.14	-.69	-1.40	-7.23	22.50	2.30	11.39
VIRGIN SOIL								
Dried blood (1 per cent).....	137.20	41.28	30.09	-0.56	-0.41	70.00	-67.20	-48.48
Vetch (2 per cent)....	65.20	7.20	11.04	-.84	-1.29	65.00	-.20	-.31
Mellilotus (2 per cent)....	48.40	11.60	23.97	13.16	27.19	74.20	25.80	53.31
Soybeans (2 per cent)....	43.40	.40	.92	7.12	16.41	45.20	1.80	4.15
Corn (2 per cent).....	30.02	.34	1.13	14.84	49.43	67.20	37.18	123.85
Cowpeas (2 per cent)....	45.00	.54	1.20	10.08	24.00	63.20	18.20	40.44
Black-eyed peas (2 per cent).....	42.40	3.20	7.55	12.88	30.38	57.40	15.00	35.38
Oats (2 per cent).....	20.20	-.72	-3.50	-1.26	-6.24	19.16	-1.04	-5.15



It is seen that the addition of 1 per cent of dried blood caused a large increase in the ammonia content of soils H, B, and virgin, a smaller increase in soil F, and a comparatively small increase in soil U. The percentage of nitrogen recovered as ammonia from the dried blood varies from 4.69 in soil U to 34.79 in soil B. There was no increase in nitric nitrogen in the virgin soil at any time during the incubation period of six weeks. On the other hand, the results indicate that there was a slight reduction of nitrates as compared with the amount found in the control. The increase in nitric nitrogen in soils H and B is very little; but in soils F and U there is a decided increase after the first 14 days, and the increase continues throughout the incubation period. In soil U 51.43 per cent of the nitrogen added in dried blood was recovered as nitrates after six weeks' incubation. In soils H, B, and virgin none of the nitrogen added as dried blood was recovered as nitrates. The loss of nitrogen from the addition of 1 per cent of dried blood varies from 13.27 per cent in soil U to 58.16 per cent in soil H.

The addition of 2 per cent of vetch caused an increase in the ammonia content of all of the soils, but the amount of increase is small as compared with the increase from dried blood. At the conclusion of the experiment the percentage of nitrogen recovered as ammonia varied from 2.58 in soil U to 20.83 in soil B. After 14 days there was an increase in nitrates in all of the soils to which vetch had been added, but the increase in the virgin soil and soil B were very small, and during the latter part of the incubation period the virgin soil, to which 2 per cent of vetch had been added, contained less nitric nitrogen than the control. In soil U 40.37 per cent of the nitrogen added in vetch was recovered as nitrates after six weeks' incubation, the virgin being the only soil in the series which failed to give less than 17.61 per cent of nitrogen as nitrates. Soil H and the virgin soil show a slight loss of total nitrogen, while soils F, B, and U show slight gains, varying from 9.51 to 18.1 per cent of the nitrogen added.

The addition of melilotus caused only a small increase in the ammonia in soils U and F, but considerable increases in soils H and B and the virgin soil. At the conclusion of the experiment the ammonia recovered from melilotus varied from 1.45 per cent in soil H to 23.97 per cent in the virgin soil. After 14 days' incubation soils U and F showed a marked increase in nitrates, soils H and B comparatively small increases, while the virgin soil showed no increase. There are gains in nitrates in all of the soils of from 14 to 42 days, and at the conclusion of the experiment the percentage of nitrogen recovered as nitrates from melilotus varies from 27.19 in the virgin soil to 84.46 in soil F. There is a decided increase in the total nitrogen in all of the soils receiving melilotus. In soil F the increase amounts to more than the nitrogen added in melilotus.

Soybeans, unlike vetch or melilotus, caused very little or no increase in the ammonia content of the soils. The increase in nitrates is also

slower than when vetch or melilotus was added. The highest percentage of nitrogen as nitrates recovered from soybeans was 30.97. The increase in total nitrogen is also comparatively low.

The addition of 2 per cent of corn gave very little or no increase in the ammonia content, and the increase in nitrates after the first 14 days was very small, and in some instances no increase was found. However, during the latter part of the incubation period the formation of nitrates took place more rapidly, and at the conclusion of the experiment 62.36 per cent of the nitrogen added in corn was recovered as nitrates in soil F, the average for the five soils being well above 50 per cent. The percentage increase in nitrogen varies from 123.3 in the virgin soil to 151.9 in soil H.

The addition of cowpeas, like the addition of soybeans and corn, caused very little or no increase in ammonia. Nitrification appears to have started somewhat slowly; but, when once started, the increase continued rather rapidly until the end of the experiment. In all of the soils the percentage of nitrogen recovered from soybeans as nitrates varies from 24 in the virgin soil to 50.4 in soil F. All of the soils show an increase in total nitrogen, although the increase is much smaller than that received from the addition of corn.

Black-eyed peas caused a moderate increase in ammonia in all of the soils. At the conclusion of the experiment the percentage of nitrogen recovered from black-eyed peas as ammonia varied from 1.33 in soil H to 7.55 in the virgin soil. There appears to have been a slight loss in nitrates at the end of 14 days in all of the soils except soil B, in which the increase amounted to only 0.14 mgm. During the latter part of the incubation period considerable quantities of nitrates were formed from black-eyed peas, and at the conclusion of the experiment the percentage of the nitrogen recovered as nitrates varied from 30.38 per cent in the virgin soil to 46.79 per cent in soil F. There was an increase in the total nitrogen in all of the soil, which varied from 18.87 per cent in soil B to 79.95 per cent in soil F.

The addition of 2 per cent of oats caused very little or no increase in the ammonia content of the soils; neither was there any increase in nitrates; but, on the other hand, the addition of oats seems to have reduced somewhat the nitrate content of all of the soils. There were small gains in total nitrogen in soils U and F, but small losses in H, B, and the virgin soils.

In the control samples the ammonia remained fairly constant throughout the experiment, but there was a slow and quite uniform increase in nitrates.

A fourth series of samples was taken from the experimental plots at Riverside on November 4, 1914, and handled similarly to those taken on August 28, except that the green manures added were generally different. The results secured in this experiment are given in Tables VIII and IX.

TABLE VIII.—*Nitrification of dried blood and green manures in Citrus Experiment Station soils, Riverside, Cal. November 4, 1914*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Material added.	Con- stituent.	Plot U.			Plot F.			Plot H.		
		Incubation period.			Incubation period.			Incubation period.		
		14 days.	28 days.	42 days.	14 days.	28 days.	42 days.	14 days.	28 days.	42 days.
Dried blood (1 per cent).	Ammonia.	17.22	18.34	16.66	43.54	47.74	28.98	47.88	49.70	40.88
Do.	Nitrates.	30.24	37.35	49.56	— .40	— .00	2.94	— .84	— 3.22	— 1.68
Sweet corn (2 per cent).	Ammonia.	.14	.00	.00	— .28	— .56	.00	.92	1.12	.56
Do.	Nitrates.	— 1.40	5.29	4.34	— 1.10	5.18	4.90	— 1.54	— 4.08	2.38
Field corn (2 per cent).	Ammonia.	— .28	.70	.00	— .14	.00	2.52	.42	.00	.28
Do.	Nitrates.	— 1.54	.67	4.76	.96	1.58	2.10	Lost.	— 5.60	1.12
Sorghum (2 per cent).	Ammonia.	— .35	.00	1.26	— .00	.14	.14	.28	.70	.28
Do.	Nitrates.	— 1.12	.11	.98	— 1.24	.28	— .84	— 1.40	— 3.78	— 1.40
Alfalfa (2 per cent).	Ammonia.	3.36	1.40	1.40	6.62	2.80	4.06	11.20	5.46	3.36
Do.	Nitrates.	18.34	23.35	28.84	6.46	12.04	11.62	3.78	3.92	13.72
Water only.	Ammonia.	1.40	1.26	1.54	1.26	1.26	1.26	1.12	.70	1.12
Do.	Nitrates.	1.96	3.25	2.40	1.66	3.22	2.38	1.96	2.60	2.66
		Plot B.			Plot C.			Plot E.		
		Incubation period.			Incubation period.			Incubation period.		
		14 days.	28 days.	42 days.	14 days.	28 days.	42 days.	14 days.	28 days.	42 days.
Dried blood (1 per cent).	Ammonia.	45.50	49.28	39.06	28.56	29.82	22.26	43.40	35.14	26.32
Do.	Nitrates.	— .70	— 3.12	.00	7.28	17.08	18.68	4.20	17.22	16.16
Sweet corn (2 per cent).	Ammonia.	.56	.56	— .70	.56	.00	.00	.00	.00	— .28
Do.	Nitrates.	— .14	.56	3.71	— 1.96	3.64	2.59	— .84	— 1.68	— 1.40
Field corn (2 per cent).	Ammonia.	.21	— .04	— .84	.28	.00	— .07	— .28	.28	.14
Do.	Nitrates.	— .14	— 4.06	1.75	— 1.96	2.66	.14	— .84	— .14	1.26
Sorghum (2 per cent).	Ammonia.	.21	— .42	— .28	.42	.56	.98	— .42	.14	— .84
Do.	Nitrates.	— .14	— 2.16	— .77	— 1.96	.14	— 2.38	— .70	— 1.12	— 1.96
Alfalfa (2 per cent).	Ammonia.	13.44	5.60	3.22	4.76	3.50	2.10	4.06	3.50	1.68
Do.	Nitrates.	2.94	16.24	13.30	6.16	12.32	14.28	3.78	18.62	14.14
Water only.	Ammonia.	.70	1.12	1.38	.84	.98	1.26	1.40	1.26	1.24
Do.	Nitrates.	.56	3.16	1.11	2.52	2.52	3.08	1.54	3.78	2.80

The addition of 1 per cent of dried blood caused a marked increase in ammonia in all of the soils. At the conclusion of the six weeks' incubation period, from 12.14 to 29.80 per cent of the nitrogen added as dried blood was recovered as ammonia. Nitrification proceeded rapidly during the first 14 days in soil U and somewhat slower in soils C and E, while soils B, F, and H showed a slight reduction in nitrates at this time. Soils B and H showed no increase in nitric nitrogen over the control at any time during the experiment and the gain in soil F amounted to only 2.94 mgm. The only soil in which the amount of nitric nitrogen exceeds the amount of ammonia recovered from the soil is soil U, and in this soil only 36.12 per cent of the nitrogen added in dried blood was recovered as nitrates. A large part of the nitrogen added as dried blood was apparently lost in all of the soils.

TABLE IX.—Percentage of nitrogen added to soils in dried blood and green manures recovered as ammonia and nitrates after six weeks' incubation; also percentage gain or loss of nitrogen added. November 4, 1914

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

PLOT U								
Material added.	Nitrogen in material added.	Nitrogen as ammonia recovered in 6 weeks.	Percent-age of nitrogen as ammonia recovered.	Nitrogen as nitrate recovered in 6 weeks.	Percent-age of nitrogen as nitrate recovered.	Nitrogen remaining in soil after 6 weeks.	Gain or loss in nitrogen.	Percent-age of nitrogen gained or lost.
Dried blood (1 per cent).....	137.20	16.66	12.14	49.56	36.12	112.00	-25.20	-18.37
Sweet corn (2 per cent).....	32.20	.00	.00	4.34	13.48	37.80	5.60	17.39
Field corn (2 per cent)	29.40	.00	.00	4.76	16.19	39.20	9.80	33.33
Sorghum (2 per cent).....	23.20	1.26	5.43	.98	4.22	36.40	13.20	56.90
Alfalfa (2 per cent)....	84.80	1.40	1.05	28.84	34.01	87.55	2.75	3.24
PLOT F								
Dried blood (1 per cent).....	137.20	28.98	21.12	2.94	2.14	66.00	-71.20	-51.90
Sweet corn (2 per cent).....	32.20	.00	.00	4.90	15.22	32.20	.00	.00
Field corn (2 per cent)	29.40	2.52	8.57	2.10	7.14	30.80	1.40	4.73
Sorghum (2 per cent).....	23.20	.14	.60	.84	3.62	24.15	.95	4.09
Alfalfa (2 per cent)....	84.80	4.06	4.79	11.62	13.70	79.50	-5.30	-6.25
PLOT H								
Dried blood (1 per cent).....	137.20	40.88	29.80	-1.68	-1.22	61.90	-75.30	-54.88
Sweet corn (2 per cent).....	32.20	.56	1.74	2.38	7.39	30.45	-1.75	-5.44
Field corn (2 per cent)	29.40	.28	.95	1.12	3.81	36.40	7.00	23.81
Sorghum (2 per cent).....	23.20	.28	1.21	-1.40	-6.04	24.50	1.30	5.60
Alfalfa (2 per cent)....	84.80	3.36	3.96	13.72	16.18	85.10	.30	.35
PLOT B								
Dried blood (1 per cent).....	137.20	39.06	28.47	0.00	0.00	64.90	-72.30	-52.70
Sweet corn (2 per cent).....	32.20	-.70	-2.18	3.71	11.52	31.85	-.35	-1.08
Field corn (2 per cent)	29.40	-.84	-2.86	1.75	5.95	29.05	-.35	-1.19
Sorghum (2 per cent).....	23.20	-.28	-1.21	-.77	-3.32	24.15	.95	4.09
Alfalfa (2 per cent)....	84.80	3.22	3.80	13.30	15.68	79.50	-5.30	-6.25
PLOT C								
Dried blood (1 per cent).....	137.20	22.26	16.22	18.68	13.62	75.60	-61.60	-44.90
Sweet corn (2 per cent).....	32.20	.00	.00	2.59	8.05	26.95	-5.25	-16.31
Field corn (2 per cent)	29.40	-.07	-.24	.14	.48	37.10	7.70	26.19
Sorghum (2 per cent).....	23.20	.98	4.22	-2.38	-10.26	22.40	-.80	-3.45
Alfalfa (2 per cent)....	84.80	2.10	2.48	14.28	16.84	80.40	-4.40	-5.19
PLOT E								
Dried blood (1 per cent).....	137.20	26.32	19.18	16.16	11.78	72.80	-64.40	-46.94
Sweet corn (2 per cent).....	32.20	-.28	-.87	-1.40	-4.35	31.15	-1.05	-3.26
Field corn (2 per cent)	29.40	.14	.48	1.26	4.29	36.05	6.65	22.62
Sorghum (2 per cent).....	23.20	-.84	-3.62	-1.96	-8.45	33.60	10.40	44.83
Alfalfa (2 per cent)....	84.80	1.68	1.98	14.14	16.67	82.60	-2.20	-2.59

The addition of 2 per cent of sweet corn caused little or no increase in the ammonia content of the soils. At the end of the first 14 days all of the soils showed a reduction in nitrates; but during the latter part of the incubation period an increase in nitrates was found in all of the soils, with the exception of soil E, which showed a slight reduction in nitrates throughout the incubation period. However, the percentage of nitrogen recovered as nitrates was comparatively low in all cases. The only gain in nitrogen from the addition of sweet corn was in soil U, in which the gain amounted to 17.39 per cent of the nitrogen added. In the other soils there is apparently a loss of nitrogen varying from 1.08 per cent in soil B to 16.31 in soil C.

The production of ammonia and nitrates from field corn was very similar to that secured from the addition of the same quantity of sweet corn; but the effect on the total nitrogen content of the soil was apparently quite different in that all of the soils, with the exception of soil B, showed considerable increase in total nitrogen, the loss in soil B being only 1.19 per cent of the nitrogen added.

The addition of sorghum frequently caused a reduction of the ammonia content of the soils, and in no case does the increase amount to more than 1.26 mgm. per 100 gm. of soil. The sorghum generally caused a reduction in the nitrate content of the soils. There is an increase in total nitrogen in all of the soils except C, in which the loss amounts to 3.45 per cent of the nitrogen added.

The addition of 2 per cent of alfalfa caused a considerable increase in the ammonia content of all of the soils. At the conclusion of the experiment from 1.65 to 4.79 per cent of the nitrogen added was recovered as ammonia. The nitrification of alfalfa proceeded rapidly during the first 14 days in soil U and more slowly in the other soils. After six weeks' incubation 13.7 per cent of the nitrogen added in alfalfa was recovered as nitrates in soil F and as much as 34.01 per cent in soil U.

In the control samples in this series, as in the early experiment, the ammonia content remained fairly uniform throughout the experiment, but there was considerable variation in the nitrates.

#### EFFECT OF TEMPERATURE AND LARGE AND SMALL APPLICATIONS OF DRIED BLOOD ON THE NITRIFYING POWER OF SEMIARID SOILS

In the nitrification experiments reported above it was found that the soils frequently failed to nitrify dried blood when it was added in 1 per cent quantities. The determination of nitric nitrogen in these soils in the field at frequent intervals showed that the application of dried blood as a fertilizer invariably increased the nitrate content of the soil. It would seem that the difference in nitrifying power exhibited by the soils in the field and under laboratory conditions was probably due to the smaller application of dried blood under the field conditions, or possibly in some measure to the difference in temperature. In order to test the



effect of temperature and also the effect of large and small quantities of dried blood upon the nitrifying power of these soils, samples of soil were collected from each of eight plots, as shown in Table X. After thoroughly mixing and removing gravel and roots, etc., six portions, each equivalent to 1 kgm. of dry soil, were weighed out from each soil.

TABLE X.—Nitrifying power of soils in incubator at 28° C. and in field at 17° to 20° C.  
March 2, 1915

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Incubation period.	Material added.	Constituent.	Plot B.	Plot T.	Plot C.	Plot S.	Plot F.	Plot O.	Plot E.	Plot U.
At beginning of experiment.		Ammonia.	0.39	0.60	0.32	0.88	0.50	0.67	0.53	0.53
		Nitrates...	.18	.18	.22	.36	.57	.43	.29	.25
After 14 days' incubation at 28° C.	No nitrogen added.	Ammonia.	.60	.62	.60	.78	.52	.72	.42	.84
	do.	Nitrates...	.48	.81	.46	.95	1.16	1.34	.46	.98
	0.1 per cent dried blood added.	Ammonia.	1.82	.18	1.40	.70	.72	.00	.00	.20
	do.	Nitrates...	6.72	6.09	6.26	7.27	7.42	6.93	7.00	7.60
	1.0 per cent dried blood added.	Ammonia.	84.70	89.04	74.20	52.22	77.56	57.94	74.20	56.28
	do.	Nitrates...	.00	.91	.00	26.11	14.00	26.65	11.62	21.21
After 28 days' incubation at 28° C.	No nitrogen added.	Ammonia.	.74	1.02	.60	1.04	.60	.88	.74	.74
	do.	Nitrates...	.53	.88	.32	1.37	1.58	1.93	.53	1.09
	0.1 per cent dried blood added.	Ammonia.	1.68	.18	.14	.14	.28	.14	.14	.14
	do.	Nitrates...	6.65	7.08	7.42	7.07	6.72	7.07	7.79	7.42
	1.0 per cent dried blood added.	Ammonia.	84.56	86.24	78.54	51.24	58.94	51.80	63.56	40.14
	do.	Nitrates...	.00	4.90	.00	33.30	28.44	30.87	24.99	49.59
After 28 days' in the field at 17-20° C.	No nitrogen added.	Ammonia.	1.02	Lost.	.74	Lost.	.74	.74	.60	.60
	do.	Nitrates...	.71	1.12	.46	1.54	1.54	1.54	.49	.83
	0.1 per cent dried blood added.	Ammonia.	1.71	2.24	.28	1.30	.00	.56	.28	.14
	do.	Nitrates...	6.33	5.92	8.26	7.42	6.86	6.86	8.22	8.68
	1.0 per cent dried blood added.	Ammonia.	61.64	36.30	71.96	43.58	33.60	26.60	44.10	28.70
	do.	Nitrates...	3.56	28.00	3.15	30.80	30.84	33.60	29.08	37.16
After 56 days' incubation at 28° C.	No nitrogen added.	Ammonia.	1.06	.84	.56	.84	.84	1.08	.68	.68
	do.	Nitrates...	1.20	2.10	1.06	2.80	2.94	3.50	1.20	2.80
	0.1 per cent dried blood added.	Ammonia.	.06	1.06	.56	.28	.28	.18	.28	.44
	do.	Nitrates...	9.72	9.94	9.99	10.20	11.76	10.08	9.44	9.94
	1.0 per cent dried blood added.	Ammonia.	63.90	64.96	65.24	49.76	55.16	34.60	50.40	39.12
	do.	Nitrates...	.47	24.78	.22	43.12	28.70	56.14	37.16	47.88
After 56 days' incubation in the field at 17-20° C.	No nitrogen added.	Ammonia.	.84	.70	.84	1.12	.84	.98	.94	.84
	do.	Nitrates...	1.24	1.68	1.26	2.24	1.96	2.35	.98	1.90
	0.1 per cent dried blood added.	Ammonia.	.24	.56	.42	.14	.28	.14	.32	.10
	do.	Nitrates...	9.22	9.24	9.94	8.68	9.24	9.27	8.66	9.02
	1.0 per cent dried blood added.	Ammonia.	75.22	55.98	84.22	46.48	41.72	38.78	50.30	41.72
	do.	Nitrates...	3.04	48.92	1.26	39.76	38.64	46.93	34.02	54.10
After 90 days' incubation at 28° C.	No nitrogen added.	Ammonia.	.32	.77	.35	.92	.53	.71	.49	.45
	do.	Nitrates...	.95	1.79	1.30	3.19	2.92	5.15	1.51	4.03
After 90 days' in the field at 17-20° C.	No nitrogen added.	Ammonia.	.46	.77	.42	1.13	.56	.71	.56	.53
	do.	Nitrates...	1.16	1.72	1.51	3.77	2.85	3.96	1.65	3.05

<sup>a</sup> Ammonia in the control not subtracted.

At the time the samples were taken the ammonia in the eight soils varied from 0.32 mgm. in soil C to 0.88 mgm. in soil S. There was little change in the ammonia content of the control samples at any time during the incubation period of 90 days, whether the samples were held in the incubator at 28° C. or in the field at 17° to 20°.

The nitric nitrogen in the soils at the time the samples were taken varied from 0.18 mgm. in soils B and T to 0.57 mgm. in soil F. After 14 days' incubation at 28°, the control samples showed an appreciable increase in nitrates. From 14 to 28 days there appears to have been little additional increase in soils B, T, C, E, and U. On comparing the nitrates in the control samples after 28 days' incubation in the two series, it appears that the increase in nitrates is about the same, regardless of the temperature at which the soils were held. From 28 to 56 days there is a rather marked increase in nitrates in the control samples in the incubator and also in the field. From 56 to 90 days there was a still further increase in nitrates in most instances; and on comparing the nitrates found in the samples held in the incubator and samples held in the field, it appears that the difference in temperature has had little effect on the increase in nitrates in these soils.

When 0.1 per cent of dried blood was added, there was an increase in the ammonia after 14 days in all of the soils except O and E. After 28 days' incubation the soils receiving 0.1 per cent of dried blood contained only a little more ammonia than the control samples. It would seem that the difference in temperature had little or no effect on the accumulation of ammonia in the soils at any time during the incubation period of 56 days, when 0.1 per cent of dried blood was added. There was a marked increase in nitrates in all of the soils receiving 0.1 per cent of dried blood after 14 days' incubation at 28°. On comparing the amount of nitrates found in the soils held at 28° and those held at from 17° to 20°, it is seen that the amount of nitrates produced from 0.1 per cent of dried blood is quite uniform in all of the soils, and it would seem that the difference in temperature has not been an influential factor in determining the rate of nitrification.

The addition of 1 per cent of dried blood caused a very large increase in ammonia. After 14 days' incubation at 28°, the ammonia varied from 56.28 mgm. in soil U to 89.04 mgm. in soil T. From 14 to 28 days there appears to have been a slight reduction in the ammonia content of some of the soils, although it is still extremely high in all cases. On comparing the amount of ammonia found in the soils held in the incubator and those held in the field soils, it would seem that the soils held in the incubator contained somewhat more ammonia. After 56 days the ammonia is still high in all of the soils, whether held in the incubator or in the field, thus indicating that nitrification has been very incomplete, even after 56 days' incubation.

The increase in nitrates from the addition of 1 per cent of dried blood after 14 days' incubation varies from 0 in soils B and C to 26.11 mgm. in

soil S. From 14 to 28 days there was an increase in nitrates in all soils except B and C, which continued to show no increase in nitrates when held at 28°; when held in the field at a temperature of 17° to 20°, these two soils showed gains of 3.56 and 3.15 mgm., respectively. After 56 days a somewhat smaller amount of nitric nitrogen was found in soils B and C than in the control samples, when held at 28°, but the increase in the other soils varies from 24.78 mgm. in soil T to 56.14 in soil O. When held at a temperature of 17° to 20° for 56 days, soils B and C showed an increase of 3.04 and 1.26 mgm., respectively. Soil T also showed a much higher nitrifying power when held at the lower temperature, but in the other soils the influence of temperature within the range of the experiment does not seem to have been an important factor.

From the data presented in Tables I to X it is obvious that the nitrifying power of a soil as determined in the laboratory by the addition of the usual amount of dried blood may be very different from that exhibited by the soil under the field conditions, and the results secured must therefore be interpreted with great care. The data presented in Table X show that, on the addition of 1 per cent of dried blood, soils B and C failed to give any increase in nitric nitrogen, even after 56 days' incubation; but, on the addition of 0.1 per cent of dried blood, these two soils nitrified as rapidly as the other soils.

It is observed that both of the virgin soils failed to nitrify 1 per cent of dried blood, while many of the soils cultivated for some years nitrified this amount of dried blood very rapidly. The power of southern California soils to nitrify 1 per cent of dried blood seems to be rather closely correlated with the character of the organic content. Those soils which have received additions of organic materials frequently nitrify 1 per cent of dried blood when the adjacent virgin soil or cultivated soils which have received no organic matter fail to give any increase in nitrates. Even those plots which have received only dried blood or bone meal frequently nitrify 1 per cent of dried blood, while the virgin soil, or soil from plots which have received no nitrifiable matter, fails to give any increase in nitrates.

In semiarid soil the growth of native vegetation is frequently very limited, owing to the meager rainfall, and the organic content of many virgin lands is consequently very low. It is well known that the proper physical, chemical, and biological characters of a soil are largely dependent upon the presence of organic matter. It therefore seems reasonable to suppose that the process of nitrification, which is so closely associated with the decomposition of organic matter, should become weakened and fail to function properly under abnormal conditions, such as are obviously produced by the large accumulation of ammonia which invariably follows the addition of 1 per cent of dried blood.

The results presented above indicate that the use of leguminous crops may be of great value not only in maintaining active organic matter in the soil but also in maintaining the nitrogen supply. In Tables I to IX

it is shown that the nitrification of green manure, especially the legumes, proceeds very rapidly. If green manures are incorporated with the soil at the proper stage of maturity, it would seem that some increase in nitric nitrogen may be expected during the first seven days and that a large percentage of the nitrogen contained in the crop will be converted into nitrates within 30 days. Early spring would seem to be the season when it is most important to have an abundant supply of available nitrogen in Citrus soils. It therefore seems inadvisable to allow the cover crop to develop until late spring, as it not only robs the tree of its nitrogen supply at a critical season but as the crop becomes more mature the nitrogen which it contains is converted into nitrates more slowly after it is plowed down. Even if a cover crop is not grown, the winter rains may carry the nitrate below the feeding roots of the trees. The rapidity of nitrification in the early spring would therefore seem to be of special importance with Citrus crops, as the nitrate content of Citrus lands is likely to be very low at that time.

The results presented above also indicate that the growth of cover crops may materially assist in maintaining the total nitrogen content of the soils. Under favorable conditions it would seem that the nitrogen gained by cover crops may more than pay for the additional cost in operation. They may also save much nitrogen from leaching away during the winter season.

In the above tables it is shown that, when 1 per cent of dried blood is added to soils, much of the nitrogen added is lost. In some cases less than 50 per cent of the nitrogen added could be recovered after six weeks' incubation. When taken from the incubator, the soils frequently gave off a strong odor of ammonia, and it is believed that much or possibly all of the loss occurred through the volatilization of ammonia. It is recognized that the determinations of the ammonia content of the soils to which 1 per cent of dried blood was added do not show the quantity of ammonia produced but rather the ammonia remaining in the soil at the time the analyses were made. Determinations of nitrites were not made, except in a few instances; but these few determinations were sufficient to show that considerable quantities of nitrites sometimes accumulated in these soils following the addition of 1 per cent of dried blood. As the reduction method was used in determining the nitric nitrogen, it is likely that some of the nitrogen recorded as nitrates may have been present as nitrites.

#### EFFECT OF FURROW IRRIGATION ON THE DISTRIBUTION OF NITRIC NITROGEN IN SOILS

In the irrigation of land many methods of applying the water are now in use; but, as a rule, the furrow system is employed in the irrigation of orchards, small fruits, root crops, and vegetables. In the irrigation of orchards the furrows may vary from 4 to 9 inches in depth, and the number of furrows run between adjacent tree rows may vary from 2 to 6. The



water which is distributed in the furrows quickly passes under the forces of gravity and capillarity. The downward movement of the water is frequently interrupted by a rather impervious plowsole; under such conditions the capillary forces cause a rapid lateral movement of the water, and in the course of a few hours the moisture may spread to all of the intervening space between the furrows. The action of the capillary forces is of paramount importance in securing an even distribution of water; but these forces, operating under the conditions mentioned above, while giving an even distribution of moisture, may have the reverse effect upon the soluble salts in the soil, especially the highly soluble salts, such as the nitrates, which possess a relatively high order of diffusibility.

The investigations in the distribution of nitrates in furrow-irrigated soils was commenced in July, 1913, and during the latter part of the season several hundred samples of soil were collected from furrow-irrigated Citrus groves in Riverside County. The analyses indicated an extremely uneven distribution of nitrates in these soils. Surface scrapings collected from brown spots, which generally occur in furrow-irrigated soils immediately after irrigation, frequently showed a nitrate content amounting to more than 0.5 per cent of nitrogen, while samples taken a few inches immediately beneath the brown spots were invariably low in nitrates and not uncommonly contained as little as 1 part per million. The very large amount of nitric nitrogen in the brown spots led to a study of the vertical distribution of the nitrates in 6-inch sections. The analyses indicated that in many groves as much as 75 per cent of the nitrate in the upper 3 feet was confined to the surface 6 inches of soil during the summer months.

Because of the frequent cultivation of Citrus groves, very few feeding roots were found in the upper 6 inches of soil. It therefore appears that the large store of available nitrogen found in the surface layers can be of little value in the nutrition of Citrus plants until carried down within reach of the feeding roots. The investigation of the vertical distribution of nitric nitrogen was continued during the winter months, and it was observed that the nitrates began to move downward as soon as the winter rains were sufficient to penetrate the soil to a greater depth than 6 inches. At the end of the rainy season the nitrates in the surface layers were extremely low. After the beginning of the rainy season there was no evidence of the brown spots, which had been so characteristic after every irrigation. However, as soon as the irrigation of the new season began, the brown coloration was again in evidence, though not so abundant as during the latter part of the previous season. Analyses of scrapings from the brown spots again showed them to be typical niter spots. During the spring and summer months extensive investigations were carried out on the nitrifying and nitrogen-fixing power of these soils. No correlation could be established between the high nitric content of the surface soil and the activity of the nitrifying or nitrogen-fixing organisms, but the field observations and preliminary laboratory studies seemed to show that there was a close correlation between the irrigation and the occur-



rence of the niter spots. The irrigation water was carefully analyzed and found to contain very small quantities of nitrates, which seemed to preclude the possibility of irrigation water being the source of the nitrates; neither did it seem at all possible that the nitrate accumulations in the Citrus soils of southern California could be derived from nitrate deposits occurring originally in the country rock. The rock from which these soils were originally derived are granitic in character, and, so far as the writer has been able to ascertain, contains little or no nitrogen. Furthermore, the virgin lands or cropped dry lands are generally very low in nitrogen and do not contain the brown spots so characteristic of furrow-irrigated lands.

TABLE XI.—Seasonal variation in nitrates in furrow-irrigated soils receiving heavy applications of nitrogenous fertilizers. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Period.	Plot A.				Plot C.				Plot F.			
Depth.....inches..	0-6	6-18	18-30	30-42	0-6	6-18	18-30	30-42	0-6	6-18	18-30	30-42
1914.												
September.....	5.18	0.74	0.46	.....	5.22	1.02	0.60	.....	2.70	0.60	0.32	.....
October.....	4.14	.46	.46	.....	4.28	.84	.46	.....	1.98	.57	.32	.....
November.....	3.60	.39	.25	.....	3.84	.97	.25	.....	1.44	.32	.18	.....
December.....	4.72	.59	.18	.....	2.61	.94	.18	.....	1.63	.81	.25	.....
1915.												
January.....	3.51	.88	.18	.....	2.72	1.02	.11	.....	1.30	.43	.29	.....
February.....	.15	.53	1.09	.....	.25	.39	1.23	.....	.25	.29	.83	.....
March.....	1.58	.88	.46	0.16	.46	.32	.25	0.82	.32	.29	.32	0.32
April.....	2.08	.88	.58	.85	.95	.39	.71	1.02	.60	.36	.25	.29
May.....	.31	.62	.51	.17	.20	.65	.37	.28	.58	.58	.22	.25
June.....	1.99	.57	.20	.29	1.77	.35	.22	.15	1.10	.22	.18	.08
July.....	3.05	.25	.29	.29	2.60	.25	.29	.11	1.30	.11	.15	.08
August.....	2.51	.22	.06	.06	3.96	.18	.10	.08	1.09	.39	.18	.11
Plot G.												
1914.												
September.....	6.62	0.74	0.46	.....	7.18	1.16	0.60	.....	5.78	0.98	0.74	.....
October.....	5.22	.74	.46	.....	6.55	.57	.74	.....	4.60	.74	1.02	.....
November.....	4.54	.66	.18	.....	4.68	.74	.40	.....	3.65	.32	.25	.....
December.....	4.17	1.13	.29	.....	4.59	1.30	.25	.....	3.75	.99	.18	.....
1915.												
January.....	3.53	.74	.18	.....	4.82	1.51	.25	.....	3.23	1.22	.15	.....
February.....	.25	1.10	1.71	.....	.22	1.03	1.74	.....	.11	1.06	1.64	.....
March.....	2.40	.66	.81	0.67	2.65	1.15	.92	0.95	1.98	.39	.74	0.99
April.....	2.76	.77	.84	.53	2.80	.60	.53	.87	2.12	.60	.71	1.02
May.....	.69	.95	.20	.13	.50	.36	.30	.49	.43	.54	.36	.57
June.....	3.52	.86	.50	.59	4.21	.78	.64	.97	2.32	.57	.64	.50
July.....	2.27	.13	.28	.04	3.33	.39	.32	.60	2.39	.64	.46	.46
August.....	4.38	.30	.78	.60	3.95	.46	.48	.56	3.54	.39	.71	1.16
Plot O.												
1914.												
September.....	2.42	0.74	0.46	.....	4.98	0.88	0.60	.....	4.70	1.30	0.74	.....
October.....	1.94	.46	.74	.....	4.42	.74	.74	.....	4.10	1.02	1.02	.....
November.....	1.04	.43	.46	.....	3.78	1.02	.32	.....	3.82	1.02	.53	.....
December.....	1.74	.66	.25	.....	3.48	1.78	.18	.....	3.05	1.79	.38	.....
1915.												
January.....	2.00	.60	.32	.....	3.57	1.23	.32	.....	3.91	1.44	.43	.....
February.....	.25	.48	.86	.....	.50	2.18	.99	.....	.67	1.79	1.16	.....
March.....	.25	.25	.67	0.54	1.85	1.02	1.30	1.09	.81	.81	1.09	1.08
April.....	.53	.36	.56	.56	2.45	1.23	1.44	.74	1.30	.81	1.10	1.08
May.....	.64	.46	.41	.36	.60	.60	.48	.34	.20	.57	.59	.67
June.....	1.41	.36	.27	.50	2.73	.85	.79	.81	2.00	1.09	.78	.95
July.....	1.79	.32	.25	.15	3.72	.46	.29	.39	3.58	.53	.60	.60
August.....	1.51	.25	.08	.18	3.12	.53	.74	.16	2.35	.29	.92	.53

In September, 1914, systematic studies were undertaken, in cooperation with the Citrus Experiment Station, to determine more accurately the distribution and movement of nitric nitrogen in Citrus soils and the factors controlling the same. Table XI shows the vertical distribution of nitric nitrogen in nine Citrus soils, receiving heavy applications of nitrogenous fertilizers.

A comparison of the amount of nitric nitrogen found in the upper 6 inches of soil in September shows that the nitrate content of the upper 6 inches is many times that found at a depth of from 6 to 18 inches or 18 to 30 inches. A similar distribution of nitric nitrogen is found in these plots during the months of October, November, December, and January. In February the quantity of nitric nitrogen in the upper 6 inches was found to be very much lower than during the previous months. The maximum amount found at this time is in plot S and amounts to 0.67 mgm. per 100 gm. of soil. In plot H the quantity in the upper 6 inches has been reduced to 0.22 mgm. It is seen that, while the amount of nitric nitrogen in the surface 6 inches has decreased, the amount at a depth of 18 to 30 inches has increased in all cases. It is therefore obvious that the nitric nitrogen has moved downward since the sampling in January. During the time intervening between the samplings the rainfall amounted to 6.52 inches, and it would seem that this precipitation was sufficient to move to below a depth of 30 inches much of the nitric nitrogen which had accumulated at the surface during the irrigation season. In March the nitric nitrogen content in the upper 6 inches is much increased in plots A, G, H, L, and Q. This increase is apparently due to the first application of fertilizer, added on February 14, which included sodium nitrate. Dried blood applied to plots C and Q or barnyard manure applied to plots F and O gives little or no increase in nitric nitrogen at this time. In April all plots show an increase in the upper 6 inches, although the gain in the manured plots is very small, indicating that the nitrogen in the manure becomes available very slowly. The plots were irrigated from April 15 to 18. Following the irrigation 2.20 inches of rain fell. It appears from the data secured that the combined effect of the irrigation and rainfall between the samplings in April and May caused a marked downward movement of the nitric nitrogen, so that none of the plots showed any concentration of nitrates in the surface 6 inches. Even at a depth of 30 to 42 inches the nitrates were much lower than during the previous month, indicating a downward movement below  $3\frac{1}{2}$  feet. The second and third applications of fertilizer were applied on May 8 and July 2, respectively. As the season advanced it was observed that there was a marked accumulation of nitrates in the upper 6 inches, but no increase in the lower layers. The distribution throughout the year indicates that the application of water in furrows has very little effect in carrying nitric nitrogen down, but that rainfall, when sufficient to penetrate the soil to considerable depth, is very effective in causing a downward movement of nitrates and other soluble salts.

Table XII shows the distribution of nitric nitrogen in soils receiving light applications of nitrogenous fertilizers, and in these soils it is seen that the accumulation of nitric nitrogen in the surface 6 inches is far less than in the heavily fertilized soils. It is also seen that the amount found at a depth of 6 to 18, 18 to 30, and 30 to 42 inches is somewhat lower than in the heavily fertilized soils. The effect of the rainfall in carrying down the nitrates is shown in the results on the lightly fertilized soils as well as on the heavily fertilized soils. The smaller percentage accumulation of nitrates at the surface during the irrigation season is probably due to the fact that all of the materials added to the lightly fertilized plots was added in one application in the early spring and plowed down, and as the amount added was not in excess of the needs of the tree, much of the nitrogen was probably assimilated before the upward movement of the soil moisture had carried it beyond reach of the roots.

TABLE XII.—Seasonal variation in nitrates in furrow-irrigated soils receiving light applications of nitrogenous fertilizers. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Period.	Plot E.				Plot N.			
Depth.....inches..	0-6	6-18	18-30	30-42	0-6	6-18	18-30	30-42
1914.								
September.....	0.88	0.46	0.52	.....	1.02	0.11	0.46	.....
October.....	.88	.57	.46	.....	1.02	.46	.32	.....
November.....	1.02	.32	.18	.....	1.14	.32	.25	.....
December.....	1.16	.53	.22	.....	1.02	.15	.18	.....
1915.								
January.....	.97	.25	.11	.....	.78	.39	.18	.....
February.....	.18	.29	.39	.....	.15	.22	.25	.....
March.....	.50	.21	.29	0.32	.58	.11	.05	0.01
April.....	1.37	.22	.18	.25	1.44	.43	.33	.24
May.....	.55	.84	.29	.13	.69	.36	.18	.11
June.....	1.30	.29	.25	.08	.61	.18	.11	.04
July.....	1.06	.22	.22	.15	1.02	.18	.15	.15
August.....	1.02	.11	.08	.08	1.05	.15	.11	.08
Plot K.					Plot P.			
1914.								
September.....	1.02	0.46	0.46	.....	1.38	0.46	0.46	.....
October.....	1.02	.46	.74	.....	1.16	.46	.47	.....
November.....	1.02	.32	.18	.....	1.14	.18	.25	.....
December.....	.99	.32	.11	.....	1.02	.46	.25	.....
1915.								
January.....	.91	.22	.11	.....	.83	.22	.11	.....
February.....	.15	.18	.60	.....	.18	.32	.28	.....
March.....	.81	.25	.32	.....	.88	.25	.11	.....
April.....	1.44	.46	.32	0.32	1.44	.53	.25	0.18
May.....	.57	.54	.25	.22	.53	.53	.34	.41
June.....	1.32	.46	.11	.04	1.08	.06	.10	.08
July.....	.99	.18	.15	.18	.61	.25	.25	.08
August.....	1.51	.36	.15	.01	1.02	.11	.11	.11

By a comparison of the results presented in Table XIII with those of Tables XI and XII the effect of the application of the nitrogenous fertilizers may be readily ascertained. The nitrate content of the heavily fertilized plots at a depth of 0 to 6 inches is far in excess of the amount found in the unfertilized plots. At a depth of 6 to 18 inches, 18 to 30 inches, and 30 to 42 inches the increase is much less marked, and in some

instances little or no increase is found. The light application of nitrogenous fertilizer has apparently increased the nitrate content of the soil very little over the unfertilized plots. This, of course, does not necessarily mean that the application of the nitrogenous fertilizers to these plots has not resulted in an increased nitrate supply, but rather that the nitrate has been assimilated almost as rapidly as formed. The character of the trees on these plots shows that the light application of nitrogenous fertilizer has resulted in the development of a larger and more thrifty tree than those produced by the control plots. The trees on the lightly fertilized plots have also produced more and better fruit.

TABLE XIII.—Seasonal variation in nitrates in furrow-irrigated soils receiving no nitrogenous fertilizers. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Period.	Plot B.				Plot D.				Plot I.			
Depth .....inches..	0-6	6-18	18-30	30-42	0-6	6-18	18-30	30-42	0-6	6-18	18-30	30-42
1914.												
September.....	0.94	0.46	0.46	.....	1.00	0.32	0.18	.....	0.74	0.46	0.18	.....
October.....	.92	.18	.32	.....	1.20	.32	.32	.....	.88	.32	.32	.....
November.....	.76	.15	.11	.....	.95	.15	.18	.....	.62	.25	.18	.....
December.....	.88	.25	.18	.....	.88	.32	.22	.....	.62	.36	.25	.....
1915.												
January.....	.75	.25	.11	.....	.76	.25	.11	.....	.57	.18	.18	.....
February.....	.11	.15	.25	.....	.15	.11	.29	.....	.11	.22	.18	.....
March.....	.11	.15	.08	.11	.18	.18	.25	.11	.17	.08	.11	0.04
April.....	.22	.08	.11	.14	.25	.18	.18	.18	.32	.11	.11	.18
May.....	.06	.34	.04	.08	.04	.29	.08	.04	.08	.29	.08	.03
June.....	.26	.13	.08	.04	.39	.04	.04	.22	.11	.03	.32	.25
July.....	.46	.11	.11	.08	.74	.18	.11	.04	.53	.11	.11	.11
August.....	.67	.04	.08	.04	.96	.15	.08	.01	1.48	.04	.04	.01
</												



The effect of rainfall in causing a downward movement of nitric nitrogen is seen to have taken place in the unfertilized plots as well as in the fertilized.

In Tables XI to XIII it was seen that the effect of the application of nitrogenous materials to the soils invariably increased the nitrate content of the soil, especially the surface layers; and it was also seen that the nitrates were very completely leached out of the surface layers by the winter rains, producing a rather marked seasonal variation in the nitrate content of the heavily fertilized soils.

TABLE XIV.—Seasonal variation in ammonia in furrow-irrigated soils receiving heavy applications of nitrogenous fertilizers. September, 1914, to August, 1915; inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Period.	Plot A.			Plot C.			Plot F.		
Depth.....inches..	0-6	6-18	18-30	0-6	6-18	18-30	0-6	6-18	18-30
1914.									
September.....	0.74	0.74	0.46	1.02	0.74	0.46	0.74	0.46	0.46
October.....	.74	.74	.46	.98	.57	.32	.98	.57	.46
November.....	.81	.18	.11	.95	.18	.15	.66	.25	.11
December.....	.74	.39	.18	.87	.50	.25	.78	.39	.32
1915.									
January.....	.60	.46	.43	.71	.57	.46	.60	.39	.22
February.....	.32	.32	.18	.46	.25	.11	.67	.11	.11
March.....	.60	.43	.29	.81	.53	.39	.60	.29	.29
April.....	.64	.16	.11	.46	.22	.15	.45	.36	.15
June.....	.39	.22	.11	.67	.22	.11	.46	.25	.08
July.....	.46	.25	.15	Lost.	.29	Lost.	.60	.22	.18
August.....	.29	.15	.11	.36	.22	.04	.36	.11	.01
	Plot G.			Plot H.			Plot L.		
1914.									
September.....	1.02	0.46	0.32	0.74	0.46	0.46	1.02	0.74	0.46
October.....	1.02	.46	.46	1.02	.74	.46	1.02	.74	.46
November.....	.88	.43	.18	.88	.39	.18	1.09	.43	.15
December.....	.74	.50	.18	.74	.39	.32	.99	.39	.29
1915.									
January.....	.57	.32	.22	.60	.43	.23	.81	.60	.25
February.....	.39	.25	.08	.53	.32	.08	.46	.25	.08
March.....	.88	.39	.25	.46	.39	.25	.60	.53	.53
April.....	.50	.18	.18	.53	.22	.22	.64	.36	.18
June.....	.60	.29	.11	.60	.29	.11	.53	.25	.04
July.....	.57	.18	.15	.59	.29	.15	.74	.26	.25
August.....	.50	.11	.08	.32	.08	.01	.43	.15	.01
	Plot O.			Plot Q.			Plot S.		
1914.									
September.....	1.02	0.74	0.60	1.30	0.46	0.32	1.30	0.74	0.46
October.....	1.02	.74	.46	1.02	.78	.57	1.14	.32	.46
November.....	.74	.25	.18	1.14	.39	.18	1.58	.46	.32
December.....	.75	.53	.46	1.20	.40	.25	1.02	.53	.15
1915.									
January.....	1.20	.50	.46	1.02	.46	.39	1.30	.46	.39
February.....	.46	.32	.08	.53	.25	.04	.60	.39	.08
March.....	.71	.46	.39	.95	.36	.18	1.09	.46	.32
April.....	.53	.39	.22	.50	.51	.36	.78	.43	.39
June.....	.67	.25	.18	.74	.43	.32	1.06	.36	.18
July.....	.67	.39	.29	.81	.25	.25	1.30	.39	.15
August.....	.71	.22	.04	.85	.18	.11	1.13	.36	.25



Tables XIV to XVI show that the ammonia content of the soils is generally highest in the upper 6 inches, regardless of whether the soils have received heavy applications, light applications, or no nitrogenous fertilizer. It is also seen that the ammonia content of the unfertilized soils is almost, if not quite, as high as the fertilized soils, which would indicate that the ammonia formed in the decomposition processes under the field conditions does not remain in the soil, as does the ammonia formed when large applications of dried blood are added in laboratory experiments. It also appears that the rainfall has little effect on the distribution of ammonia, and a large number of data collected in later studies have shown that the lateral movements of the irrigation water, while it caused a very uneven distribution of nitrates, had very little effect on the distribution of ammonia. A comparison of the data presented in Tables XIV and XVI shows that the nitrogen as ammonia in the unfertilized soils is frequently greater than the nitrogen as nitrates; and, as the ammonia seems to be quite uniformly maintained throughout the year, its value as a source of nitrogen for Citrus plants becomes a matter of importance.

TABLE XV.—Seasonal variation in ammonia in furrow-irrigated soils receiving no nitrogenous fertilizers. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Period.	Plot B.			Plot D.			Plot I.		
Depth.....inches..	0-6	6-18	18-30	0-6	6-18	18-30	0-6	6-18	18-30
<b>1914.</b>									
September.....	0.74	0.74	0.46	0.46	0.46	0.46	0.74	0.46	0.46
October.....	.74	.57	.46	.46	.46	.46	.57	.46	.18
November.....	.81	.25	.18	.10	.18	.25	.53	.53	.46
December.....	.69	.32	.18	.46	.39	.18	.67	.36	.25
<b>1915.</b>									
January.....	.67	.46	.43	.67	.53	.29	.67	.39	.22
February.....	.46	.25	.11	.39	.25	.11	.60	.46	.18
March.....	.46	.32	.36	.60	.39	.18	.74	.39	.25
April.....	.25	.22	.18	.39	.22	.11	.50	.29	.18
June.....	.36	.11	.04	.53	.29	.08	.60	.39	.15
July.....	.29	.25	.08	.39	.22	.15	.46	.32	.11
August.....	.22	.11	.08	.25	.08	.01	.29	.18	.01

Period.	Plot J.			Plot M.			Plot R.			Plot T.		
Depth.....inches..	0-6	6-18	18-30	0-6	6-18	18-30	0-6	6-18	18-30	0-6	6-18	18-30
<b>1914.</b>												
September.....	0.74	0.60	0.46	0.74	0.60	0.46	0.88	0.74	0.32	0.88	0.60	0.46
October.....	.74	.46	.46	.88	.57	.57	.86	.46	.32	.74	.46	.46
November.....	.81	.67	.25	.74	.32	.25	1.09	.60	.39	.88	.74	.32
December.....	.74	.46	.32	.85	.74	.46	1.09	.39	.25	.85	.36	.18
<b>1915.</b>												
January.....	.88	.67	.22	.67	.53	.25	.88	.39	.36	.67	.39	.32
February.....	.60	.43	.18	.53	.25	.08	.53	.25	.08	.53	.32	.04
March.....	.81	.36	.25	.74	.53	.39	.55	.39	.25	.81	.46	.32
April.....	.50	.40	.29	.50	.74	.29	.57	.43	.36	1.00	.60	.37
June.....	.74	.39	.18	.43	.18	.25	.60	.46	.32	.67	.18	.15
July.....	.67	.39	.11	.60	.39	.18	.57	.25	.22	.46	.25	.15
August.....	.43	.18	.01	.50	.15	.01	.43	.18	.01	.39	.22	.15

TABLE XVI.—Seasonal variation in ammonia in furrow-irrigated soils receiving light applications of nitrogenous fertilizers. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Period.	Plot E.			Plot N.			Plot K.			Plot P.		
Depth.....inches..	0-6	6-18	18-30	0-6	6-18	18-30	0-6	6-18	18-30	0-6	6-18	18-30
1914.												
September.....	0.74	0.46	0.46	0.88	0.60	0.46	0.88	0.70	0.46	0.88	0.46	0.46
October.....	.74	.57	.57	.74	.46	.46	.74	.46	.46	.88	.46	.18
November.....	.60	.22	.18	.74	.39	.15	.67	.88	.18	.81	.39	.18
December.....	.67	.46	.18	.99	.50	.39	.74	.88	.25	.88	.46	.39
1915.												
January.....	.78	.50	.22	.71	.60	.29	.67	.46	.32	.81	.43	.36
February.....	.53	.11	.11	.46	.29	.08	.60	.39	.15	.46	.32	.08
March.....	.78	.50	.22	.63	.53	.25	.88	.43	.36	.79	.43	.15
April.....	.81	.18	.22	.99	.54	.25	.60	.29	.08	.60	.29	.15
June.....	.67	.25	.25	.74	.39	.18	.67	.32	.18	.53	.22	.15
July.....	.50	.22	.11	.60	.36	.18	.46	.29	.18	.67	.25	.18
August.....	.39	.15	.07	.44	.22	.01	.29	.11	.04	.50	.22	.08

In the preliminary studies on the distribution of nitric nitrogen under the furrow system of irrigation it was observed that the supply of nitrates near the surface was reduced near the furrows during an irrigation. However, no increase in nitrates could be detected in the deeper layers to account for the reduction which apparently took place near the surface. Before and after the June irrigation of 1915, samples were drawn, 9 inches from the furrows, from 10 plots. Each sample for analysis was made up of six borings, each one located 9 inches from a furrow. The holes made in removing the first samples were carefully filled in, and the second set of borings was made about 12 inches from the first, but the same distance from the furrows.

In order to bring out more clearly the seasonal variation and the effect of fertilizers on the nitrate and ammonia content of furrow-irrigated Citrus soils, the data presented in Tables XI to XVI are summarized in Tables XVII and XVIII.

TABLE XVII.—Average nitrate content of Citrus Experiment Station soils. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Treatment and depth in inches.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.
Seven plots receiving 145 pounds of nitrogen per acre in commercial fertilizers:												
0-6.....	5.67	4.77	3.98	3.77	3.62	0.31	1.68	2.07	0.42	2.65	2.99	3.40
6-18.....	.97	.73	.73	1.22	1.15	1.15	.75	.75	.61	.72	.38	.34
18-30.....	.60	.70	.32	.23	.23	1.37	.80	.84	.40	.54	.40	.54
30-42.....							.82	.87	.38	.61	.36	.45
Two plots receiving approximately 145 pounds of nitrogen per acre as barnyard manure:												
0-6.....	2.56	1.96	2.14	1.69	1.65	.25	.29	.57	.61	1.25	1.55	1.30
6-18.....	.67	.52	.37	.74	.51	.38	.27	.36	.51	.29	.22	.32
18-30.....	.39	.53	.32	.25	.30	.85	.50	.40	.31	.23	.20	.13
30-42.....							.43	.42	.30	.29	.11	.15
Four plots receiving 48.60 pounds of nitrogen per acre in commercial fertilizer:												
0-6.....	1.08	1.02	1.08	1.05	.87	.16	.69	1.42	.58	1.08	.92	1.15
6-18.....	.37	.49	.29	.37	.27	.25	.21	.41	.57	.25	.21	.18
18-30.....	.48	.50	.22	.19	.12	.38	.19	.27	.24	.14	.19	.11
30-42.....							.17	.25	.22	.06	.14	.07
Seven plots receiving no nitrogenous fertilizers:												
0-6.....	.92	.97	.84	.80	.70	.15	.23	.40	.10	.34	.61	.70
6-18.....	.50	.38	.22	.34	.22	.25	.19	.30	.31	.07	.20	.09
18-30.....	.36	.40	.16	.18	.13	.23	.18	.26	.11	.11	.13	.07
30-42.....							.19	.20	.08	.12	.13	.04

TABLE XVIII.—Average ammonia content of Citrus Experiment Station soils. September, 1914, to August, 1915, inclusive

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Treatment and depth in inches.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	June.	July.	Aug.
Seven plots receiving 145 pounds of nitrogen per acre in commercial fertilizers:											
0-6.....	1.02	0.99	1.05	0.90	0.80	0.47	0.78	0.58	0.66	0.74	0.55
6-18.....	.61	.62	.35	.45	.47	.29	.44	.30	.29	.27	.18
18-30.....	.42	.46	.18	.23	.34	.09	.31	.23	.14	.18	.09
Two plots receiving approximately 145 pounds of nitrogen per acre as barnyard manure:											
0-6.....	.88	1.00	.70	.76	.90	.57	.66	.49	.57	.63	.54
6-18.....	.60	.66	.25	.40	.45	.22	.37	.38	.25	.30	.16
18-30.....	.53	.40	.14	.39	.34	.10	.34	.19	.13	.23	.03
Four plots receiving 48.60 pounds of nitrogen per acre in commercial fertilizer:											
0-6.....	.85	.78	.71	.82	.74	.51	.77	.75	.65	.56	.41
6-18.....	.56	.49	.47	.58	.50	.28	.47	.33	.30	.28	.18
18-30.....	.46	.42	.17	.30	.30	.11	.25	.18	.19	.16	.05
Seven plots receiving no nitrogenous fertilizers:											
0-6.....	.74	.71	.71	.76	.73	.52	.67	.53	.56	.49	.36
6-18.....	.60	.49	.47	.43	.48	.32	.41	.42	.28	.30	.16
18-30.....	.44	.41	.30	.26	.30	.11	.28	.25	.17	.12	.04

On comparing the quantity of nitric nitrogen in the surface 6 inches before and after irrigation in Table XIX, it is observed that the amount found in the second set of samples is less in every plot. The average reduction for the 10 plots amounts to a little less than 30 per cent. There is also a consistent reduction at a depth of 6 to 18 inches, which amounts to nearly 40 per cent. The average nitrate content of the plots before irrigation at a depth of 18 to 30 inches is 0.471 mgm.; after the irrigation the average for the 10 plots was only 0.272 mgm. At a depth of 30 to 42 inches the nitrate content is still somewhat lower after irrigation. The averages for all plots indicate that some movement of nitrates may have taken place at a depth of 42 to 54 inches. Below a depth of 54 inches the difference between the two sets of samples would seem to indicate that the irrigation had had little or no effect upon the distribution of the nitrates. Certainly there is no indication that the nitrates in the surface layers have been carried down into the deeper layers by the irrigation.

Since the determination of nitric nitrogen in the samples drawn 9 inches from the furrows before and after the June irrigation had shown a reduction in nitrates in the surface layers without any apparent increase in the deeper layers, it was believed that the irrigation must have caused a lateral movement, which would result in an increase in the nitrate content of the surface soil at some point farther from the furrows, presumably about midway between the furrows, as the water moving laterally from adjacent furrows would be most likely to meet near this point. As the furrows were run about 36 inches apart, samples were drawn from each of eight plots 9 inches from the furrows and 18 inches

from the furrows immediately before and as soon after the October irrigation as the moisture condition would permit. The results secured in the experiment are shown in Table XX.

TABLE XIX.—Vertical distribution of nitric nitrogen before and after the June irrigation of 1915

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Depth, in inches.	Sampled before or after irrigation.	Plot.										Average of all plots.
		A.	C.	T.	G.	H.	I.	O.	Q.	S.	U.	
0-6.....	Before.....	1.99	1.70	1.10	3.52	4.21	2.32	1.41	2.73	2.00	2.21	2.399
	After.....	1.39	1.10	1.01	2.74	2.25	1.58	.90	1.62	1.97	1.97	1.659
6-18.....	Before.....	.57	.35	.22	.86	.78	.57	.36	.85	1.09	1.34	.699
	After.....	.22	.25	.13	.60	.29	.38	.30	.67	.53	.77	.414
18-30.....	Before.....	.20	.22	.18	.50	.64	.64	.27	.79	.78	.49	.471
	After.....	.15	.08	.06	.42	.18	.29	.18	.62	.39	.35	.272
30-42.....	Before.....	.29	.15	.08	.59	.97	.50	.50	.81	.95	.36	.520
	After.....	.30	.21	.14	.46	.80	.49	.49	.50	.62	.40	.441
42-54.....	Before.....	.15	.15	.08	.78	.97	.37	.42	.71	1.02	.23	.488
	After.....	.21	.18	.14	.69	.68	.35	.35	.67	.70	.23	.420
54-66.....	Before.....	.11	.22	.25	.46	.94	.64	.29	1.34	.85	.20	.530
	After.....	.16	.18	.30	.49	.82	.66	.35	1.40	.84	.27	.537
66-78.....	Before.....	.15	.20	.11	.64	.71	.98	.49	1.34	1.13	.15	.590
	After.....	.24	.18	.16	.53	.86	1.14	.49	1.08	.90	.21	.579
78-90.....	Before.....	.20	.25	.20	.53	.78	.60	.29	.92	.60	.13	.450
	After.....	.18	.27	.14	.72	.57	.64	.45	.81	.63	.15	.456
90-102.....	Before.....	.25	.18	.20	.42	.72	.29	.32	.78	.59	.15	.390
	After.....	.28	.28	.20	.38	.69	.50	.29	.74	.46	.15	.397
102-114.....	Before.....	1.17	.22	.18	.18	.53	.29	.....	.99	.42	.16	.414
	After.....	.69	.29	.18	.35	.49	.39	.....	.67	.42	.16	.364

TABLE XX.—Distribution of nitrates before and after the October irrigation of 1915

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Depth in inches.	Sampled before or after irrigation.	Plot A.		Plot C.		Plot F.		Plot G.	
		9 inches from furrow.	18 inches from furrow.	9 inches from furrow.	18 inches from furrow.	9 inches from furrow.	18 inches from furrow.	9 inches from furrow.	18 inches from furrow.
0-6.....	Before.....	1.66	1.34	3.28	3.13	1.37	1.55	2.89	2.74
	After.....	.53	3.40	.71	13.60	.46	3.12	2.21	8.08
6-18.....	Before.....	.30	.15	.40	.53	.16	.50	.46	1.23
	After.....	.06	.39	.13	1.44	.15	.65	.15	2.63
18-30.....	Before.....	.06	.32	.15	.19	.08	.38	.03	.57
	After.....	.29	.44	.16	1.10	.18	.60	.03	1.13
30-42.....	Before.....	.11	.25	.15	.15	.09	.23	.08	.57
	After.....	.22	.46	.08	.59	.11	.69	.11	1.82
		Plot H.		Plot I.		Plot O.		Plot Q.	
		9 inches from furrow.	18 inches from furrow.	9 inches from furrow.	18 inches from furrow.	9 inches from furrow.	18 inches from furrow.	9 inches from furrow.	18 inches from furrow.
0-6.....	Before.....	5.19	5.33	2.70	3.72	1.18	.79	1.58	3.51
	After.....	.81	18.41	1.09	24.44	.53	2.20	1.40	6.78
6-18.....	Before.....	.57	1.39	.25	.92	.20	.36	.22	1.55
	After.....	.38	1.46	.13	.81	.18	1.42	.32	1.66
18-30.....	Before.....	1.30	1.92	.16	1.29	.25	.11	.67	1.18
	After.....	1.09	2.56	.07	2.42	.27	.85	.74	3.54
30-42.....	Before.....	1.24	1.31	.18	.39	.23	.22	.55	.20
	After.....	.95	3.51	.36	3.40	.27	.53	.99	.93

In the surface 6 inches the nitrates 9 inches from the furrows are higher in all of the eight soils before irrigation, the reverse being true of the samples taken 18 inches from the furrows. As the results are consistent



throughout the entire series of eight plots, it would seem that there must have been a movement of nitrates away from the furrows, causing a reduction near the furrows and an increase 18 inches from the furrow. At a depth of 6 to 18 inches the nitrates were apparently moved more slowly; however, the amount found 9 inches from the furrows is somewhat less after irrigation in all the soils except soil Q, while at a distance of 18 inches from the furrow there is a gain in all of the soils with the exception of soil L. At a depth of 18 to 30 inches the nitric nitrogen is very low, and no consistent reduction is shown in the samples taken 9 inches from the furrow; but a consistent gain is shown in samples 18 inches from the furrow, indicating that a lateral movement has taken place at this depth. The results secured at a depth of 30 to 42 inches are very similar to those obtained for a depth of 18 to 30 inches.

During the season of 1916 extensive studies were carried out on the lateral movement of nitric nitrogen in furrow-irrigated soils. At the beginning of the irrigation season small squares were selected in each of 14 plots. Samples were drawn from these squares just before and as soon after irrigation as the moisture conditions would permit. One set

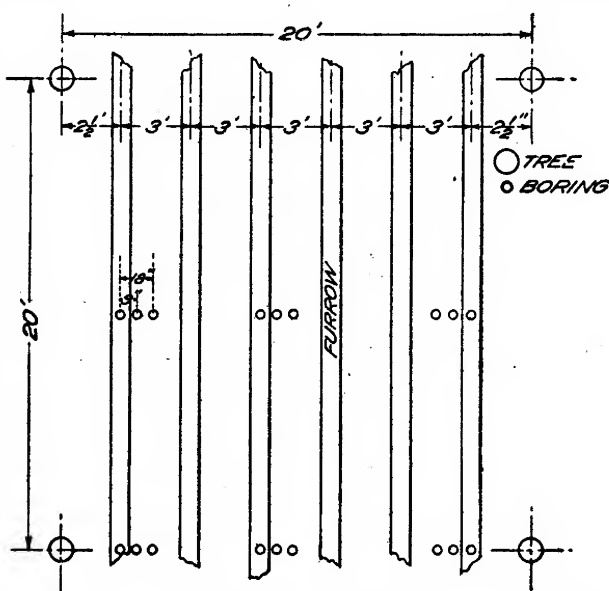


FIG. 2.—Diagram showing the location of soil-sample borings made in studying the lateral movement of nitrates in furrow-irrigated soils.

of samples was taken from the bottom of the furrows, a second set about 9 inches from the furrows, and a third set about 18 inches from the furrows. The furrows are approximately 36 inches from center to center; therefore the third set of samples were drawn from a point about midway between the furrows. Each sample for analysis was made up from six borings, which were located within the square formed by four trees. The distribution of the borings within the square is shown in figure 2. The samples were drawn from the same square before and after each irrigation. The holes made in taking the first set of samples were filled in and the borings made in securing the second set of samples were located as near the first borings as practicable. As the furrows were about 6 inches deep and the samples drawn after the furrows were run



out, the results for the upper 6 inches show only the nitric nitrogen and 18 inches from furrows. Figure 3 shows the distribution of nitric nitrogen in the soil of plot A, beginning with the first irrigation and

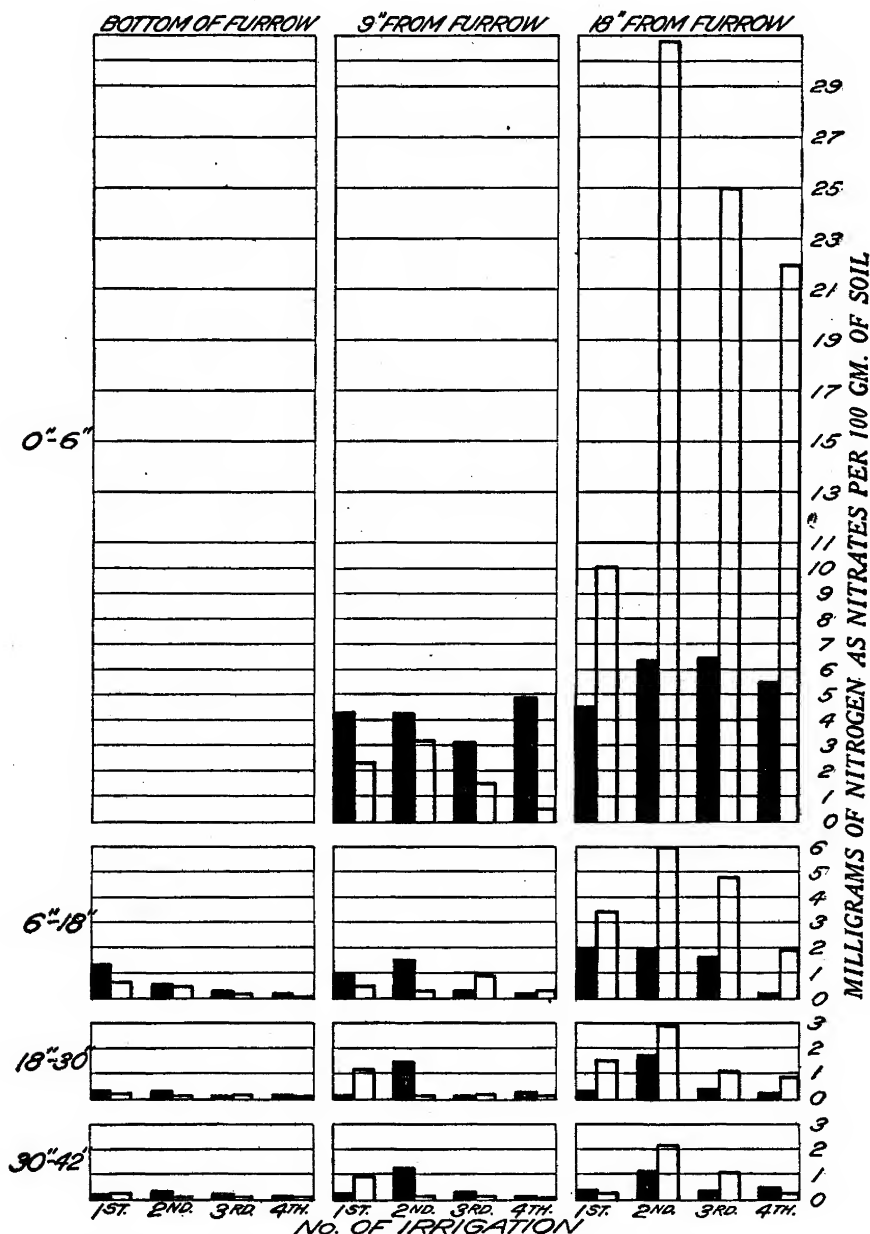


FIG. 3.—Diagram showing the distribution of nitrates in plot A before and after irrigations. Season of 1916.

continuing for four successive irrigations. The samples drawn just before the first irrigation show a rather even distribution of nitrates in this soil, which in the upper 6 inches amounts to about 4.5 mgm. After

irrigation the nitrates 9 inches from the furrow have been reduced to about 2.5 mgm., while the soil 18 inches from the furrows has increased to 10 mgm. A lateral movement has also taken place at a depth of 6 to 18 inches. The results obtained below a depth of 18 inches for the first irrigation are somewhat erratic. Samples drawn before the second irrigation indicate a more even distribution of nitric nitrogen than did the samples drawn after the first irrigation. In the upper 6 inches the frequent cultivation between irrigations is, no doubt, a factor in bringing about a more even distribution of nitrates. However, it is seen that there is apparently a more even distribution of nitric nitrogen below the cultivated zone before each irrigation than after the previous irrigation. It does not seem possible that the greater uniformity in distribution before irrigations can be due to diffusion, as the moisture in soils, when not water-soaked, is quite different from that existing in the case of liquids confined in a vessel. The soil moisture being distributed in discontinuous phases would seem to make the force of diffusion of little consequence in bringing about a more even distribution of the nitrates concentrated in zones by the lateral movement of the irrigation water. It is believed that the apparently more even distribution before an irrigation than after the last irrigation may be explained in a large measure by the lack of uniformity in the distribution of furrows from one irrigation to another. It is readily apparent that if the position of the furrows varied a few inches from one irrigation to another the samples drawn 9 inches from the new furrows might be equidistant between the old furrows, or possibly less than 9 inches from an old furrow. As the samples for analysis were made up of six borings, it seems reasonable to suppose that the results before irrigation would indicate a more even distribution of nitrates than the samples drawn after the last irrigation unless the location of the furrows were run at exactly the same point for each irrigation.

The second irrigation showed a very marked lateral movement, which was apparent even at a depth of 30 to 42 inches. Before irrigation the nitric nitrogen amounted to about 4.5 mgm. 9 inches from the furrow and 6.5 mgm. 18 inches from the furrow. After irrigation the nitric nitrogen 9 inches from the furrow was reduced to 3.2 mgm., while the amount 18 inches from the furrow was increased to 30.8 mgm. The lateral movement was also marked in the second, third, and fourth depths. The effect of the third and fourth irrigations, like the first and second, caused marked changes in the lateral distribution of the nitric nitrogen. During the fourth irrigation the supply in the upper 6 inches 9 inches from the furrow fell from 4.9 to 0.5 mgm., while the supply equidistant between the furrows rose from 5.5 to 21.9 mgm.

Plot B has received no fertilizer, and the nitrate supply was very low. However, a comparison of the nitrate content 9 inches from the furrow with that 18 inches from the furrow showed that there has apparently

been a strong lateral movement of nitrates in the surface layers, causing a variation from 0.18 to 5.85 mgm. (fig. 4).

At the beginning of the irrigation season the soil of plot C, as shown in figure 5, had a fairly uniform lateral distribution of nitric nitrogen. During the irrigation season the distribution of nitrates was very uneven in this soil.

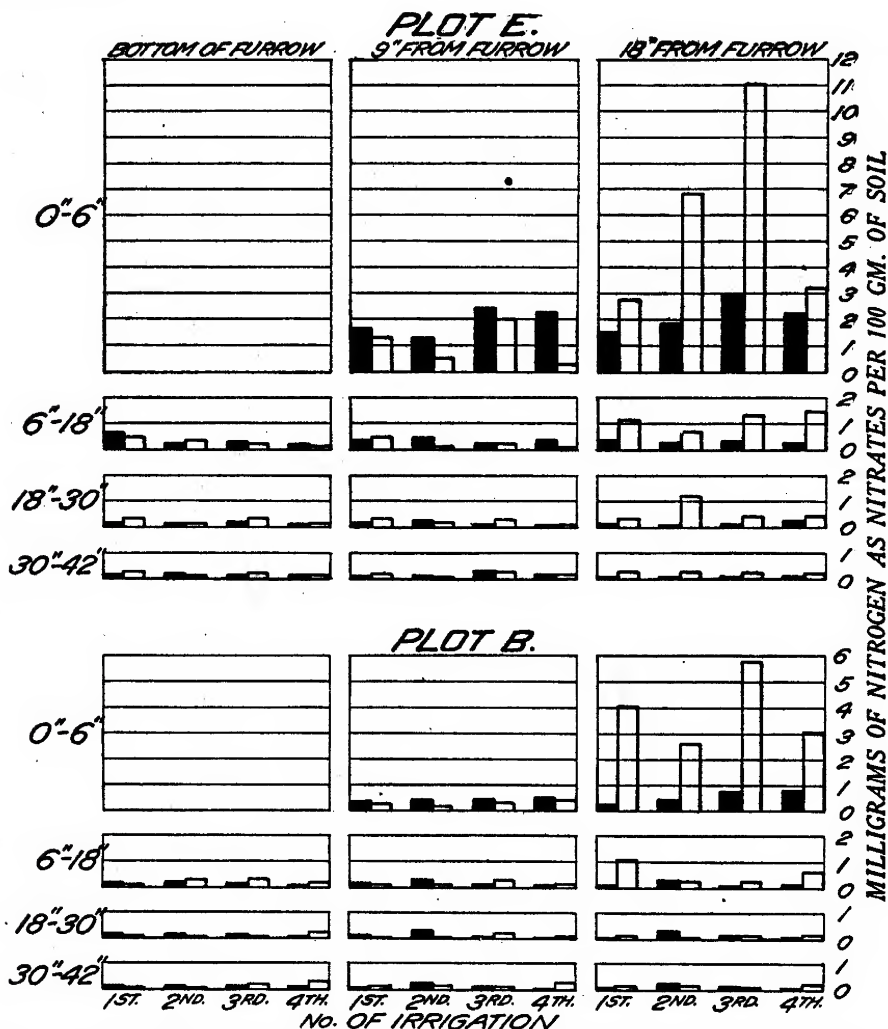


FIG. 4.—Diagram showing the distribution of nitrates in plots E and B before and after irrigations. Season of 1916.

Plot E received only one-third as much nitrogen as plot A, and it was observed that the quantity of nitrate accumulating between the furrows was much smaller. However, in the upper 6 inches the lateral movement was consistent throughout the season and showed a variation from 0.29 to 11.1 mgm. The lowest amount of nitric nitrogen found at a depth of 6 to 18 inches was 0.08 mgm., and the maximum amount was 1.45 mgm.

At a depth of 18 to 30 inches the nitrate content was very low in all cases, the maximum amount found being only 0.32 mgm.

The nitrate content of the surface 6 inches in plot F as shown in figure 6 amounted to only 1.2 mgm. at the time of the first irrigation. During

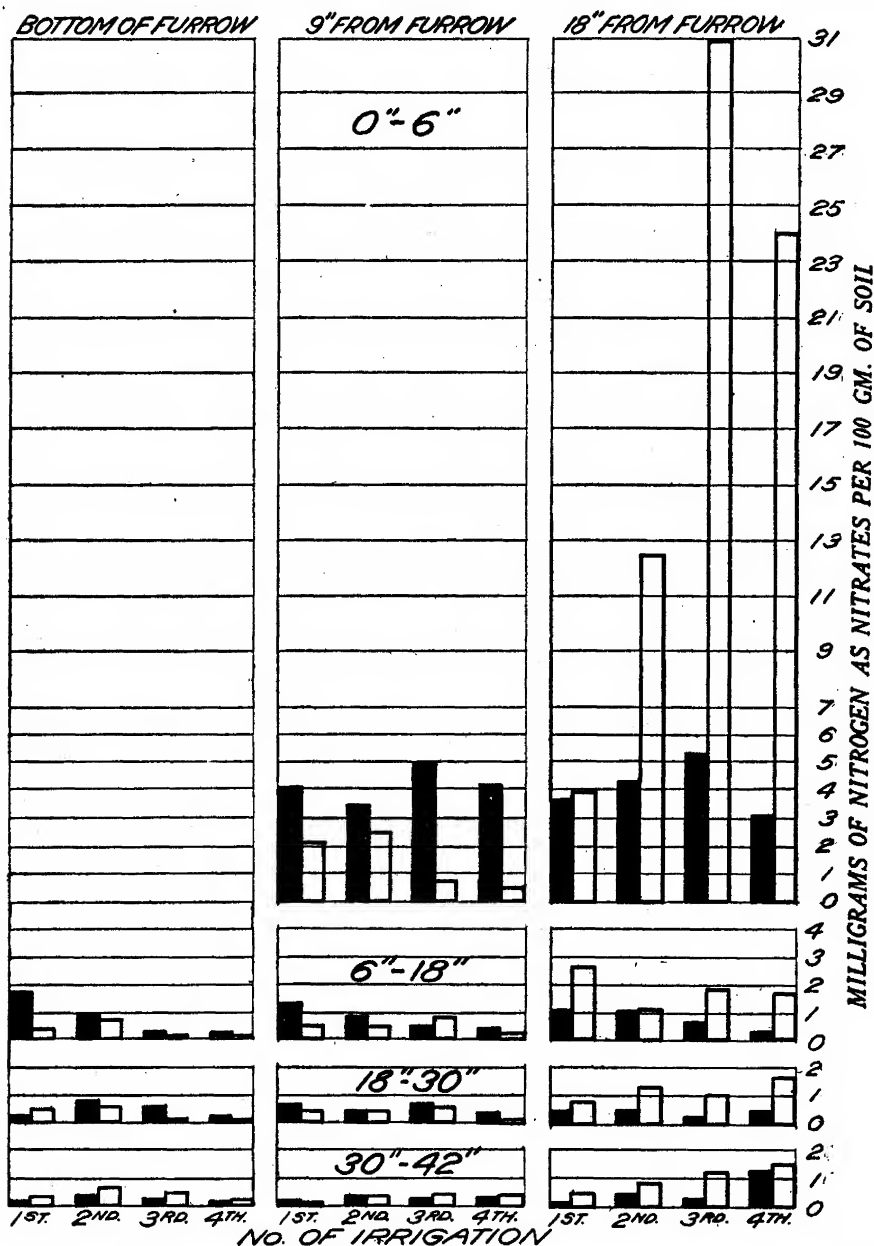


FIG. 5.—Diagram showing the distribution of nitrates in plot C before and after irrigations. Season of 1916.

the time intervening between the first and second irrigation the nitric nitrogen increased to 2.48 mgm., after which the amount remained fairly constant for the remainder of the season. The increase between the first

and second irrigation was presumably due to the nitrification of the barnyard manure, which was applied several weeks before the first irrigation. It was observed that the concentration of nitric nitrogen in the surface 6 inches of soil 18 inches from the furrow was comparatively small, considering the amount of nitric nitrogen in the soil. In this soil the highest nitrate content amounted to 6.55 mgm., which is but little higher than the highest amount secured in plot B, which received no nitrogenous fertilizers and in which the nitrate content as a whole was much lower. Notwithstanding the comparatively weak lateral movement in the surface 6 inches, the concentration of nitrates 18 inches from the lower depth was quite marked, causing a very uneven distribution of the nitrates within reach of the feeding roots.

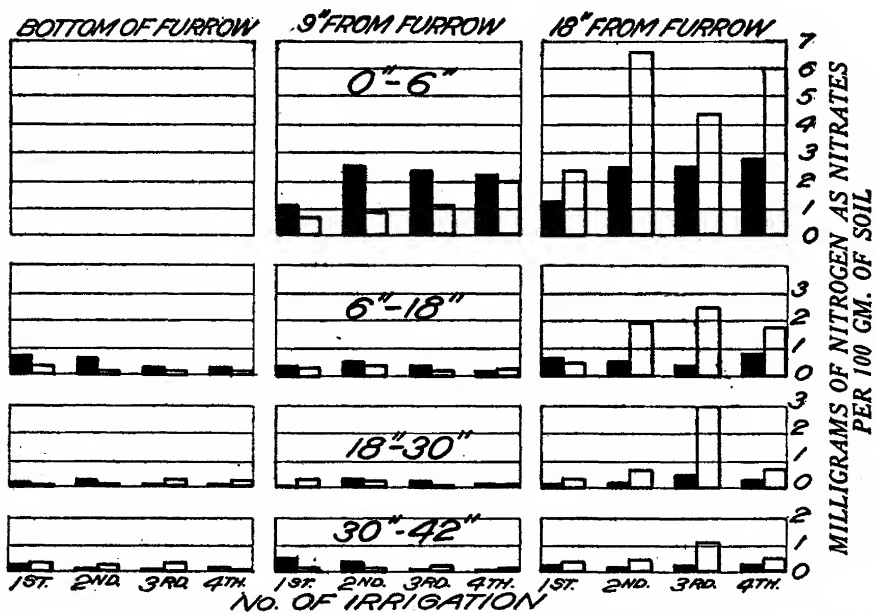


FIG. 6.—Diagram showing the distribution of nitrates in plot F before and after irrigations. Season of 1916.

Plot G shows an accumulation of nitric nitrogen 18 inches from the furrows after the second irrigation, amounting to 29.65 mgm., while the supply 9 inches from the furrow at this time amounted to only 2.56 mgm. At the lower depths the nitrate content is much smaller, and the variation is less marked. However, there is a tendency for the nitrates to move toward the point farther from the furrows, even at a depth of 30 to 42 inches (fig. 7).

At the beginning of the irrigation season the soil of plot H showed a high nitrate content in the upper layers, which amounted to 6.22 mgm. in the upper 6 inches and 3.63 mgm. at a depth of 6 to 18 inches. Before the first irrigation the nitric nitrogen in the upper 6 inches was found to be nearly the same at 9 and 18 inches from the furrow. After the third irrigation the variation was from 2.14 to 42 mgm. The distribution



of the nitric nitrogen is also extremely variable at the lower depth, but the lateral movement of the nitrates from the furrows is not as consistently shown in the soil of plot H as in some of the other soils (fig. 8).

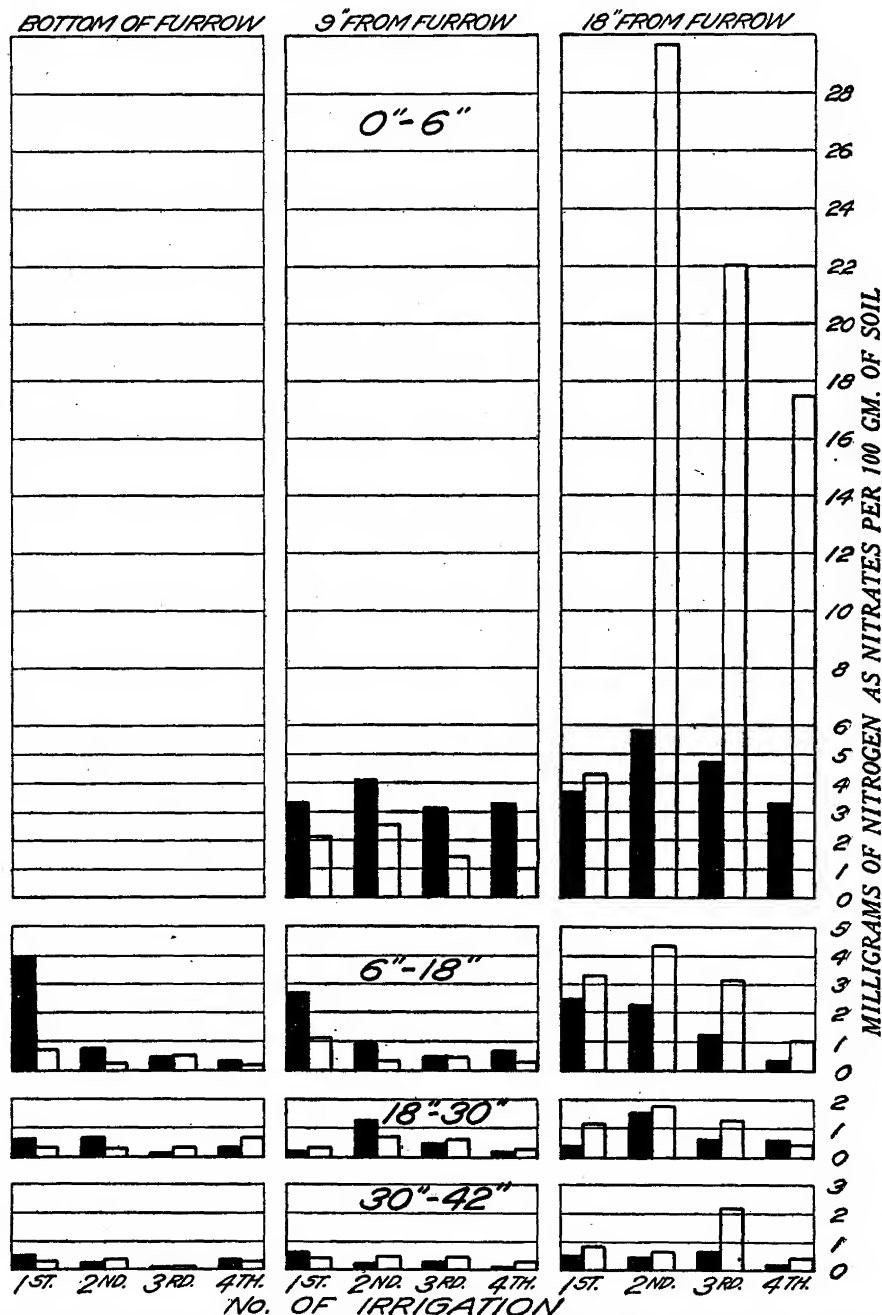


FIG. 7.—Diagram showing the distribution of nitrates in plot G before and after irrigations. Season of 1916.

The data presented in figure 9 show a strong and rather consistent lateral movement of nitric nitrogen in soil L. In the upper 6 inches the variation during the season was from 0.69 to 37.28 mgm. From 6 to 18

inches the variation was from 0.11 to 4.08 mgm. The next depth shows a variation from 0.11 to 3.75 mgm.; and even then at a depth of 30 to 42 inches the variation during the season was from 0.18 to 2.14 mgm.

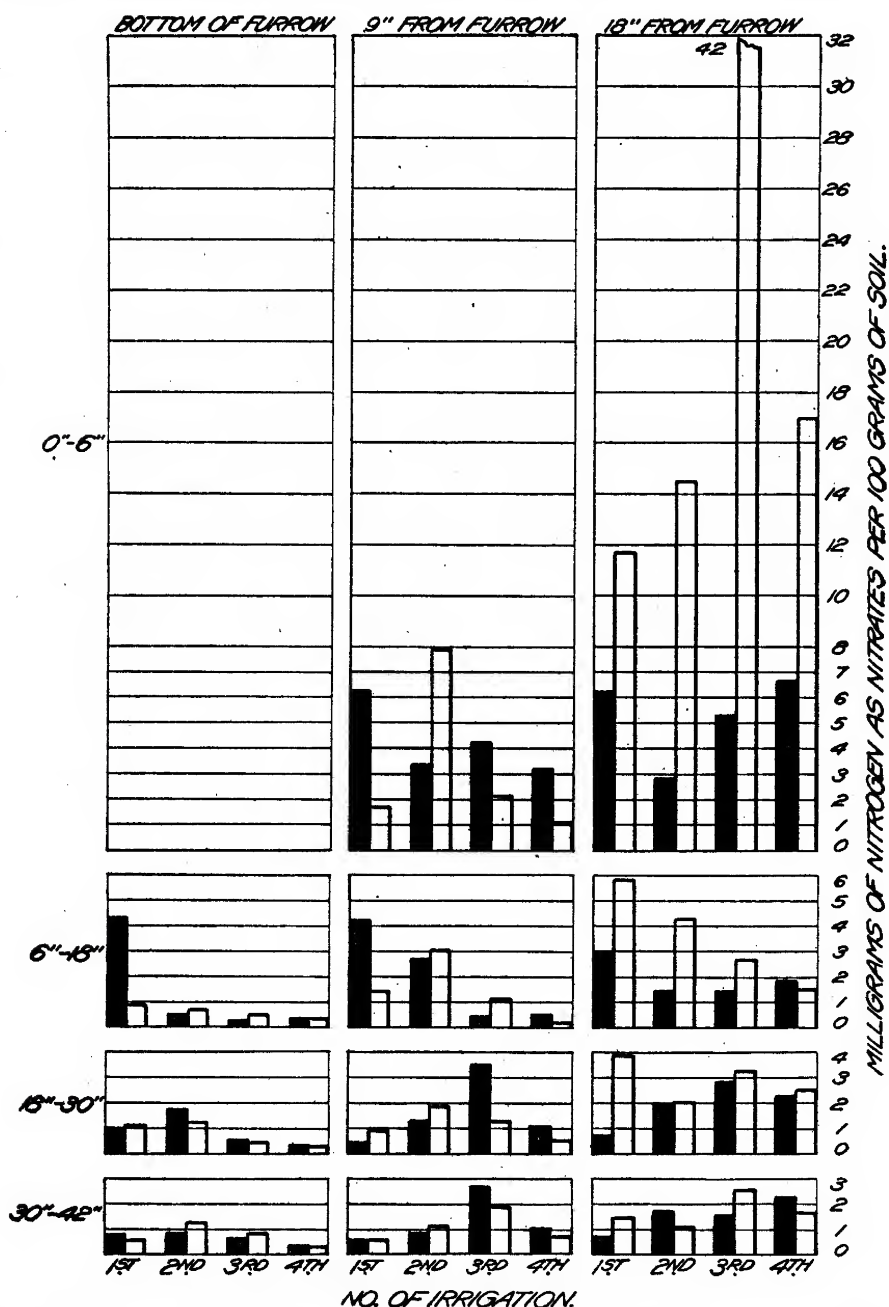


FIG. 8.—Diagram showing the distribution of nitrates in plot H before and after irrigations. Season of 1916.

Plot M has never received any nitrogenous fertilizers; consequently the nitrate content of the soil is extremely low. Even the upper 6 inches showed a nitrate content of only 0.55 mgm. at the time of the first irri-

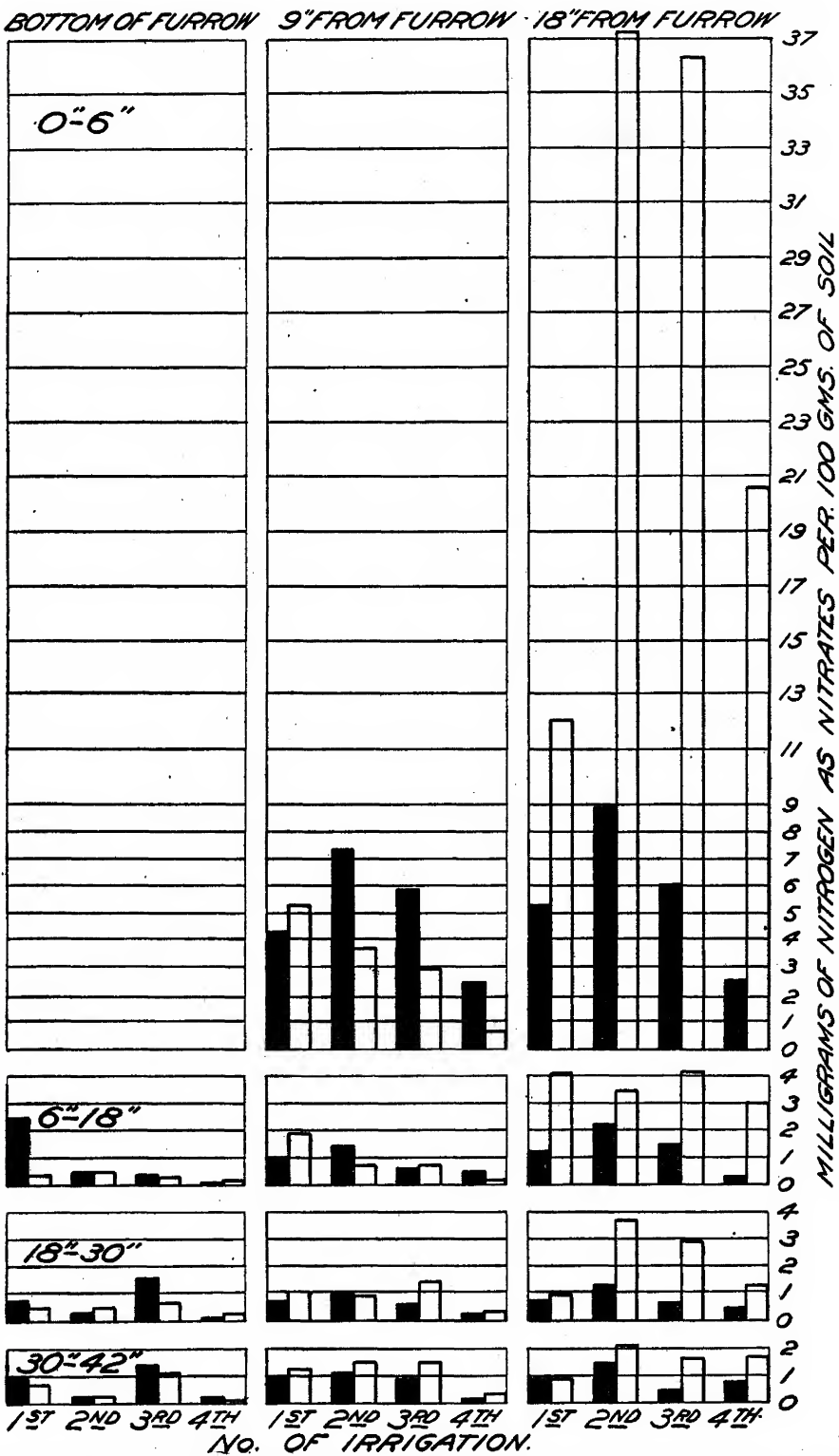


FIG. 9.—Diagram showing the distribution of nitrates in plot L, before and after irrigations. Season of 1916.

gation. With this small nitrate content it would seem scarcely possible to secure high concentrations at any point as a result of the irrigation. However, after the second irrigation the nitric nitrogen in the surface 6 inches of soil 18 inches from the furrow amounted to 4.07 mgm., while 9 inches from the furrows it amounted to only 0.5 mgm. The nitrate supply below 6 inches is very low, and little or no movement can be traced to the irrigation (fig. 10).

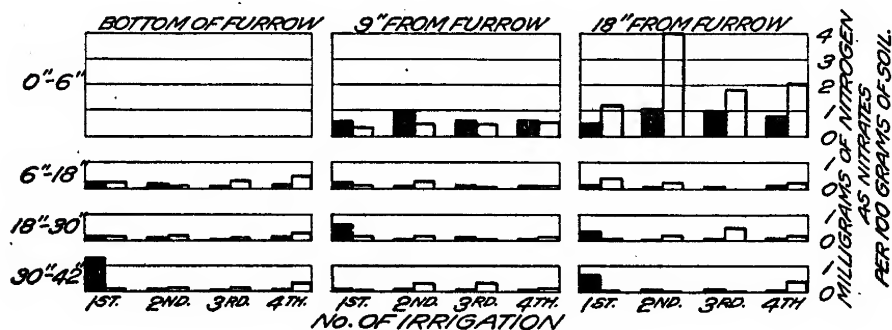


FIG. 10.—Diagram showing the distribution of nitrates in plot M before and after irrigations. Season of 1916.

The nitric nitrogen in the soil from plot O was low at the beginning of the irrigation season. The upper 6 inches showed a supply of only 0.65 mgm., while the amount of the lower depths was considerably less. Between the first and second irrigations there is a rather marked increase in the nitrate content of the upper 6 inches. This increase, like that in

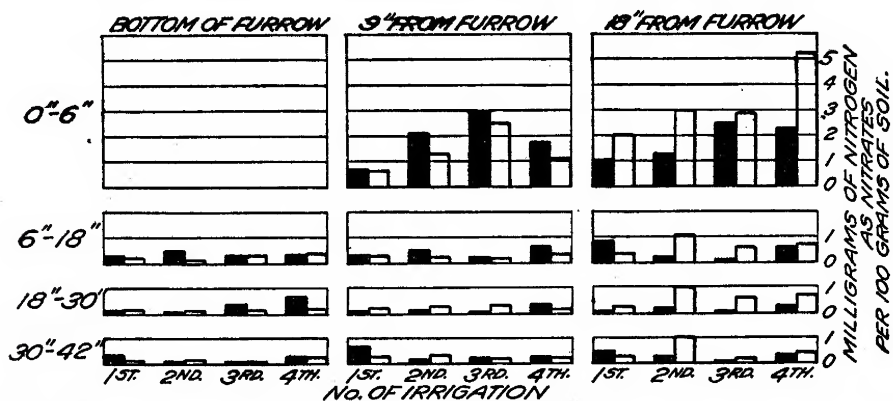


FIG. 11.—Diagram showing the distribution of nitrates in plot O before and after irrigations. Season of 1916.

plot F, is presumably due to the nitrification of the manure which was applied during the early spring. The maximum amount of nitric nitrogen found in the upper 6 inches of this soil was 5.25 mgm., which indicates a very weak lateral movement. There was apparently some tendency for the nitrates to move away from the furrows in the deeper layers, but the distribution was apparently much more uniform than in the other manure plot, which was a much lighter soil (fig. 11).

At the time of the first irrigation the nitrate content of the upper 6 inches of soil Q amounted to 3.07 mgm. In this soil the maximum nitrate content of 17.81 mgm. was secured in the upper 6 inches after the third irrigation. The highest nitrate content at each depth was found at the point farthest from the furrows, while the lowest in each depth was

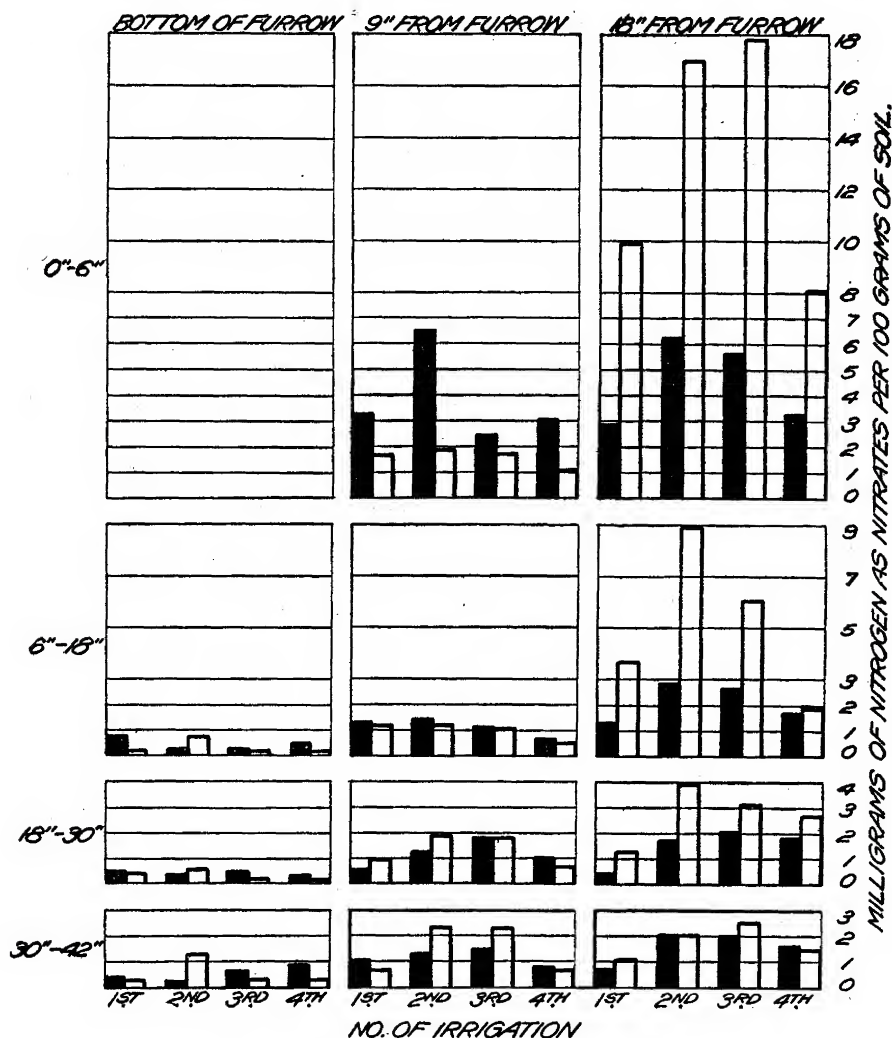


FIG. 12.—Diagram showing the distribution of nitrates in plot Q before and after irrigations. Season of 1916.

below the furrows. However, the minimum amount under the furrows was in no case less than 0.25 mgm., and the minimum amount 9 inches from the furrow was 0.56 mgm. Thus, it would seem that while the nitrate supply in this soil is somewhat uneven, there is no part of the soil which does not contain a considerable amount of available nitrogen (fig. 12).



The nitrate content of the soil in plot R, like the other soils which have received no nitrogenous fertilizers, is very low. At the time of the first irrigation the amount found in the upper 6 inches amounted to only 0.34 mgm. The maximum quantity found within reach of the roots amounted to only 0.21 mgm., and in many cases less than 0.10 mgm. was found. After the second irrigation the nitric nitrogen in the upper 6 inches 18 inches from the furrows amounted to 2.5 mgm., while only 0.29 mgm. was found 9 inches from the furrows (fig. 13).

Plot S lies immediately adjacent to plot R, and the effect of the dried blood added has evidently caused a very marked increase in the nitrate content of the soil. At the time of the first irrigation the upper 6 inches contained 6.36 mgm., the layer from 6 to 18 inches 2.19 mgm., the layer from 18 to 30 inches 1.10 mgm., and the layer from 30 to 42 inches 1.09 mgm. Thus, it is seen that at the beginning of the irrigation season there was a very satisfactory nitrate supply in this soil, and that the lateral distribution was apparently quite uniform before the first irri-

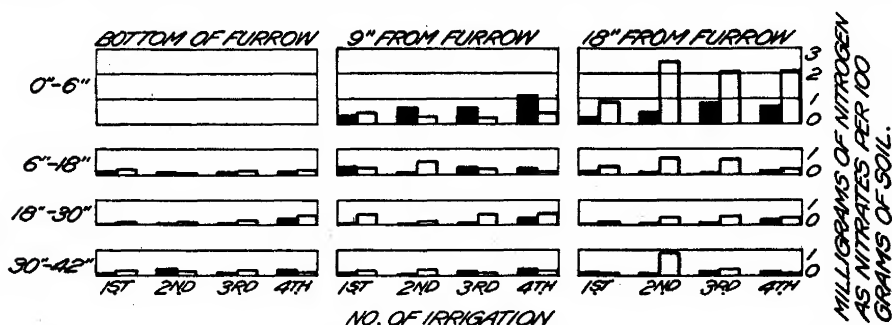


FIG. 13.—Diagram showing the distribution of nitrates in plot R before and after irrigations. Season of 1916.

gation, but quite uneven during the irrigation season. However, the nitric nitrogen showed a more satisfactory distribution in this soil during the irrigation season than in plot C, which received the same treatment, but which was a lighter soil (fig. 14).

At the time of the first irrigation the nitric nitrogen in plot U amounted to 1.31 mgm. in the upper 6 inches, 0.52 mgm. at a depth of 6 to 18 inches, and at the lower depths to less than 0.20 mgm. During the irrigation season the amount of nitric nitrogen found in the upper 6 inches varied from 0.88 to 11.8 mgm. At a depth of 6 to 18 inches the maximum amount found amounted to 3.05 mgm., while the minimum amount found at this depth was 0.25 mgm. (fig. 15).

The data presented in figures 3 to 16 show conclusively that the furrow system of irrigation causes a very uneven distribution of nitric nitrogen in soils; but as the work progressed it became apparent that samples drawn from the bottom of the furrows, 9 and 18 inches from the furrows, were not sufficient to show the maximum effect of the irrigation, as the highest concentration of nitrates did not always occur midway

between the furrows. As the highest concentration could not always be located from observation, it seemed necessary, in order to secure an accurate knowledge of the distribution between furrows, to remove all of the soil in small blocks from one furrow to another. A special soil sampler was therefore designed, which made it possible to remove a block of soil 2 by 4 inches to any depth desired. As it was evident that a very high percentage of the nitrogen accumulated in the surface layers, the soil was removed in 3-inch layers. Each block of soil 2 by 4 by 3

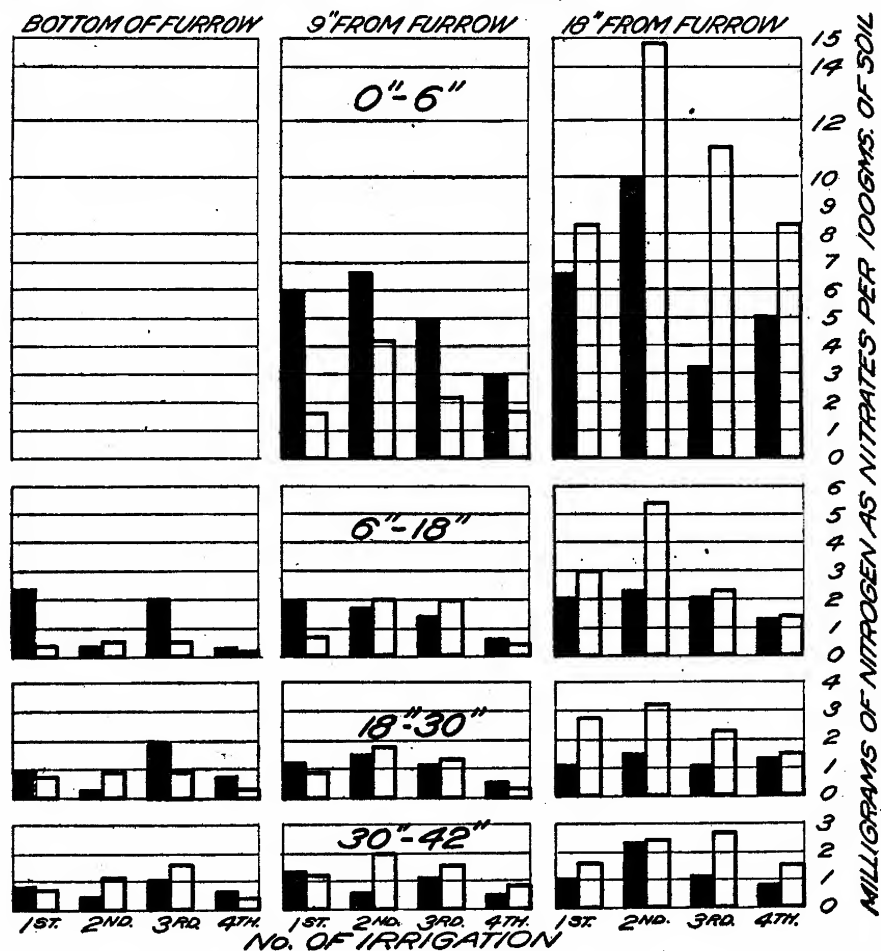


FIG. 14.—Diagram showing the distribution of nitrates in plot S before and after irrigations. Season of 1916.

inches was analyzed separately. In some instances the soil below a depth of 12 to 15 inches was removed in 12-inch sections, a number of borings being made in the bottom of the trench to make up the sample for analysis.

On August 7 samples were taken from five plots in the Citrus Experiment Station grove, as shown in Table XXI. All of the soil was removed in a 2-inch strip from furrow to furrow to a depth of 12 inches in plots A, B, G, and M, and to a depth of 15 inches in plot L.

TABLE XXI.—Distribution of nitrates at right angles to furrows, Citrus Experiment Station grove, Riverside, Cal. August 7, 1916

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Plot No.	Depth.	Boring No.									Average.
		1	2	3	4	5	6	7	8	9	
A.....	Inches.										
	0-3	2.60	3.58	12.08	13.41	29.30	21.00	20.41	7.40	7.33	13.01
	3-6	.61	.89	1.23	1.16	3.89	2.00	1.34	.64	.19	1.33
	6-9	.57	.57	.32	.95	2.00	1.30	1.38	.43	.68	.91
	9-12	.29	.50	.88	1.79	1.51	1.02	1.48	.64	.61	.97
B.....	0-3	.28	.42	1.06	1.58	1.06	.67	.32	.....	.....	.78
	3-6	.18	.15	.08	.64	.18	.11	.15	.....	.....	.21
	6-9	.15	.18	.08	.15	.11	.11	.18	.....	.....	.14
	9-12	.08	.18	.08	.11	.08	.08	.08	.....	.....	.10
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.....	.08
G.....	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.....	.15
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.....	.15
	0-3	.43	3.58	6.66	4.35	35.01	19.61	5.47	1.41	.89	8.60
	3-6	.40	3.96	.99	2.39	10.23	1.87	.36	.29	.15	2.29
	6-9	.33	.36	.26	.92	3.74	2.27	.50	.19	.19	.97
L.....	9-12	.43	.36	.36	1.27	2.04	.99	.40	.29	.29	.72
	0-3	2.10	11.80	23.10	67.90	21.49	4.50	2.50	1.50	.90	15.09
	3-6	.27	1.10	1.96	3.75	6.08	5.46	.56	.28	.26	2.19
	6-9	.24	2.00	2.10	.95	2.80	5.20	.79	.28	.24	1.62
	9-12	.25	.20	1.85	1.20	2.20	1.49	1.20	.40	.18	1.00
M.....	12-15	.21	.21	.44	1.00	.98	.78	.60	.36	.21	.53
	0-3	.28	.30	4.10	4.40	1.10	.76	.49	.28	.....	1.46
	3-6	.20	.28	.56	.65	.42	.40	.28	.28	.....	.38
	6-9	.20	.56	.84	.56	.30	.48	.20	.18	.....	.42
	9-12	.18	.18	.21	.28	.41	.35	.26	.24	.....	.26

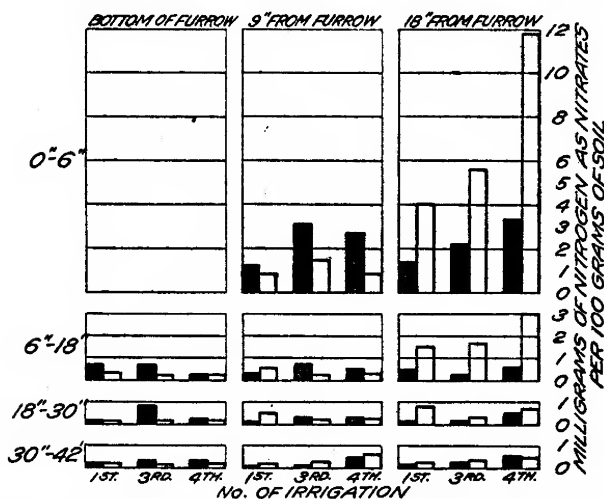


FIG. 15.—Diagram showing the distribution of nitrates in plot U before and after irrigations. Season of 1916.

In plot A it is observed that the highest amount of nitric nitrogen found in the upper 3 inches of soil was in boring 5—29.30 mgm.—while the amount found in the surface 3 inches in boring 1 amounted to only 2.60 mgm. The highest amounts found in the second and third sections were also for boring 5, which was about midway between the furrows. It is

therefore apparent that the distribution of nitrates in this soil was influenced to a considerable extent by the irrigation. The column of averages for plot A shows that 80 per cent of the nitrates in the upper foot was found in the surface 3 inches, and the next highest amount was at a depth of 3 to 6 inches.

The samples from plot B were taken between furrows run at a distance of 28 inches. Only seven borings were therefore necessary to remove the

soil between the furrows. Soil B never received any fertilizer and, as shown in figure 1, it lies adjacent to soil A, which received 145.8 pounds of nitrogen per acre each year. On comparing the amount of nitric nitrogen found in plot B with that in plot A, it is seen that soil A contained about 18 times as much nitric nitrogen as soil B in the upper 3 inches. At a depth of 3 to 6 inches soil B contained only 0.21 mgm., as compared with 1.33 mgm. at the same depth in soil A. Soil B also contained a much smaller nitrate content from 6 to 12 inches than did soil A, thus showing that the fertilizer applied to soil A very greatly increased the nitrate content of the surface layers of the soil.

In the upper 3 inches of soil G the variation in nitrate content was from 0.43 mgm. in boring 1 to 35.01 mgm. in boring 5. The column of averages shows that a very high percentage of the nitrates found in the first foot of this soil was confined in the upper 6 inches, which are above the feeding roots of the tree.

The results secured with the soil in plot L, were similar to those obtained in soils A and G, although the highest nitrate content was not always midway between the furrows, indicating that the lateral movement of the irrigation water took place more rapidly from one furrow than from the other.

The amount of nitrates found in the soil of plot M, like that of plot B, was low. However, the distribution of the nitrates was quite variable. The highest amounts are always found at some distance from the furrows; but, as in soil L, the maximum quantity found is not always midway between the furrows. Although no fertilizer was applied to this soil, there was a tendency for the nitrates to accumulate in the upper 3 inches, where the average amount was 1.46 mgm., as compared with 0.38 mgm. at a depth of 3 to 6 inches.

On September 12 and 13 samples were drawn from five plots in the Citrus Experiment Station grove at Arlington. The distribution of nitrates in these soils is given in Table XXII.

Plot 13 has received 22 pounds and 14 ounces of blood per tree each year for two years. The nitrate in the upper 3 inches on September 12, 1916, is shown in Table XXII. The soil removed in borings 2 and 6, which are near the furrows, contains only 3.37 and 2.28 mgm., respectively, while the soil removed in boring 4, which is farthest from the furrows, contains 40.78 mgm. A study of the column of averages is interesting, as it indicates that about 50 per cent of the nitric nitrogen found in the first 4 feet of soil is located in the upper 3 inches, which is well above the feeding roots of the tree. It also appears that the lowest nitrate supply is from 6 to 24 inches, at which depths the largest number of feeding roots are probably located.

Plot 14 lies immediately adjacent to plot 13 and was conducted as a control. The character of the soil was quite similar, and, as the samples in both plots were taken between furrows 28 inches apart, it would seem that the difference in nitrate content may be attributed to

the fertilization. On comparing the column of averages for these two plots it is seen that the nitrate content of plot 14 was small as compared with that found in plot 13. But, notwithstanding the limited supply of nitrates in this soil, it is seen that approximately 75 per cent of the nitric nitrogen found to a depth of 4 feet was confined in the upper 3 inches of soil. In plot 14 the maximum amount found at each depth was in boring 4, which was farthest from the furrows, indicating a consistent lateral movement of the nitric nitrogen.

TABLE XXII.—*Distribution of nitrates at right angles to furrows, Citrus Experiment Station grove, Arlington, Cal.*

[Results expressed as milligrams of nitrogen per 100 gms. of soil]

Plot No., material added, and sampling date.	Depth.	Boring No.								Average.
		1	2	3	4	5	6	7	8	
13 (dried blood), sampled Sept. 12, 1916.	<i>Inches.</i>									
	0-3	.....	3.37	27.90	40.78	11.38	2.28	.....	.....	17.14
	3-6	0.64	5.22	3.61	3.19	1.20	1.69	1.27	.....	2.40
	6-9	.22	.39	1.37	1.16	.46	.43	.36	.....	.63
	9-12	.29	.39	.78	1.86	.53	.36	.60	.....	.69
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.43
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	1.51
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	1.30
	0-3	.....	.99	4.77	8.51	1.44	.46	.....	.....	3.23
	3-6	.29	.25	.43	.81	.25	.25	.18	.....	.35
14 (control), sampled Sept. 12, 1916.	6-9	.11	.22	.22	.46	.11	.18	.15	.....	.21
	9-12	.08	.11	.25	.46	.11	.18	.11	.....	.19
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.05
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.05
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.04
	0-3	.....	.59	6.94	7.78	5.26	4.81	.73	.....	4.35
	3-6	.43	.49	1.43	.89	.85	.61	.38	0.29	.67
	6-9	.22	.28	.64	.71	.78	.71	.43	.64	.55
	9-12	.22	.33	1.77	.32	.39	.61	.50	.19	.54
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.29
26 (control), sampled Sept. 12, 1916.	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.15
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.22
	0-3	.....	3.23	12.12	12.76	32.00	13.10	8.62	.....	13.64
	3-6	.54	.99	.85	3.97	6.17	1.10	.71	1.06	1.92
	6-9	.22	.64	.40	1.59	6.73	1.06	.43	.43	1.44
	9-12	.22	.29	.26	.82	4.42	1.90	.33	.19	1.05
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.43
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.43
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.12
	0-3	.....	9.88	88.70	9.66	3.86	.....	.....	.....	28.01
25 (barley-straw mulch and nitrate of lime), sampled Sept. 13, 1916.	3-6	1.62	2.15	7.26	1.06	1.06	.75	.....	.....	2.31
	6-9	1.73	.71	1.95	1.94	1.10	1.24	.....	.....	1.44
	9-12	1.83	.99	3.72	1.34	1.20	.78	.....	.....	1.64
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	2.64
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	2.39
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	1.62
	0-3	.....	9.88	88.70	9.66	3.86	.....	.....	.....	28.01
	3-6	1.62	2.15	7.26	1.06	1.06	.75	.....	.....	2.31
	6-9	1.73	.71	1.95	1.94	1.10	1.24	.....	.....	1.44
	9-12	1.83	.99	3.72	1.34	1.20	.78	.....	.....	1.64
36 (alfalfa hay), sampled Sept. 13, 1916.	12-24	.....	.....	.....	.....	.....	.....	.....	.....	2.64
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	2.39
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	1.62
	0-3	.....	9.88	88.70	9.66	3.86	.....	.....	.....	28.01
	3-6	1.62	2.15	7.26	1.06	1.06	.75	.....	.....	2.31
	6-9	1.73	.71	1.95	1.94	1.10	1.24	.....	.....	1.44
	9-12	1.83	.99	3.72	1.34	1.20	.78	.....	.....	1.64
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	2.64
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	2.39
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	1.62

Plot 25 was kept covered with barley straw, but even under a mulch of this character it is seen that there was a large accumulation of nitric nitrogen at the surface, which varied from 3.23 to 32.0 mgm. in the upper 3 inches. The results also indicate a strong lateral movement to a depth of 12 inches. The highest nitrate content in each case was secured in boring 5, which was presumably the point at which the water met. The column of averages indicates that little nitric nitrogen has been carried below a depth of 3 feet and that at least two-thirds of the nitric nitrogen in the first 4 feet was confined to the surface 3 inches.

Plot 26 lies immediately adjacent to plot 25 and has received no fertilizer of any character during the last two years. The samples from this plot were taken between furrows 32 inches apart; and it is



observed that there is no one boring which was consistently high, but that there was an appreciable movement of nitrates away from the furrows. The nitrate supply within reach of the roots was higher in this plot than in plot 14, which was also a control. Nearly 50 per cent of the nitrates in the upper 4 feet of soil were found in the upper 3 inches.

In plot 36 the samples were taken between the furrows run at a distance of 24 inches. The nitrate content of the surface 3 inches varied from 3.86 to 88.7 mgm. The highest amount found below the surface 3 inches is 7.26 mgm. Notwithstanding the large accumulation of nitrates in the surface soil, it is seen that the nitrate supply in this soil within reach of the feeding roots was abundant. The increase at the lower depth was presumably due to the leaching down by the winter rains of the nitrates produced during the previous season.

It would seem that the fertilized plots in this grove contained enough available nitrogen above the feeding roots to supply the needs of the trees from 1½ to 3 years. Even the unfertilized soils contained more nitrogen above the feeding roots than would be removed in an average crop of fruit.

In order to determine the distribution of nitric nitrogen in soils differing in type and treatment, samples were taken in representative groves from widely separated districts. Table XXIII shows the distribution of nitrates in soils from Covina, Corona, and Lordsburg.

TABLE XXIII.—*Distribution of nitrates at right angles to furrows in soils at Covina, Corona, and Lordsburg, Cal.*

[Results expressed as milligrams of nitrogen per 100 gms. of soil]

Locality and sampling date.	Depth.	Boring No.								Average.
		1	2	3	4	5	6	7	8	
	<i>Inches.</i>									
Covina (sampled Aug. 9, 1916).....	0-3	6.73	11.47	7.29	13.66	6.52	2.74	.....	.....	8.07
	3-6	0.14	.74	1.86	1.69	2.18	.93	.50	0.32	1.04
	6-9	.81	.57	.66	.90	.67	.33	.30	.29	.57
	9-12	.25	.18	.17	.45	.32	.24	.38	.22	.28
	12-15	.46	.60	.29	.60	.39	.27	.34	.25	.40
Corona (sampled Aug. 14, 1916).....	0-3	7.95	10.89	21.88	21.18	17.96	9.56	7.57	1.09	12.26
	3-6	.99	1.09	5.15	28.18	5.57	7.11	1.51	.43	6.25
	6-9	.84	.67	1.37	3.75	4.59	3.40	.81	.35	1.97
	9-12	1.02	.57	.29	.88	1.02	.95	.95	.28	.74
	12-15	.56	.43	.25	.39	.67	.74	.64	.25	.49
Lordsburg, soil A (sampled Aug. 23, 1916).....	0-3	.....	.74	7.60	20.27	1.65	3.12	.39	.....	5.03
	3-6	.25	.88	.67	1.72	.53	.67	.15	.36	.65
	6-9	.67	.50	.25	.92	.32	.18	.50	.25	.45
	9-12	.25	.15	.50	.64	.25	.25	.18	.18	.30
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	1.58
Lordsburg, soil B (sampled Aug. 23, 1916).....	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.46
	0-3	.....	18.94	10.75	9.84	14.88	12.29	17.54	.....	14.04
	3-6	5.82	6.34	3.09	6.65	3.16	8.69	4.42	3.75	5.24
	6-9	1.44	1.93	1.41	1.41	1.76	2.28	1.51	.85	1.57
	9-21	.....	.....	.....	.....	.....	.....	.....	.....	.39
Lordsburg, soil C (sampled Aug. 23, 1916).....	21-33	.....	.....	.....	.....	.....	.....	.....	.....	.32
	0-3	.....	17.54	25.64	24.64	20.34	16.00	12.08	.....	19.34
	3-6	1.09	1.16	4.52	4.73	2.60	2.14	.43	.78	2.18
	6-9	.53	.57	.78	.81	.46	.39	.46	.25	.53
	9-12	.22	.32	.32	.25	.25	.18	.18	.18	.24
Lordsburg, soil D (sampled Aug. 23, 1916).....	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.15
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.18
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.25
	0-3	.....	9.21	10.68	20.90	14.74	1.30	.....	.....	11.37
	3-6	.29	.60	1.76	4.10	.85	1.13	2.14	.....	1.55
Lordsburg, soil D (sampled Aug. 23, 1916).....	6-9	.60	.39	.32	.60	.46	.39	.32	.....	.44
	9-21	.....	.....	.....	.....	.....	.....	.....	.....	1.27
	21-33	.....	.....	.....	.....	.....	.....	.....	.....	.18
	33-45	.....	.....	.....	.....	.....	.....	.....	.....	.39

The samples taken from the grove at Covina were taken between furrows which were run at a distance of 32 inches. The nitrates in the upper 3 inches varied from 2.74 mgm. in boring 7 to 13.66 mgm. in boring 5, showing that there was a lateral movement of nitric nitrogen away from the furrows. From 3 to 6 inches the nitrates varied from 0.14 mgm. in boring 1 to 2.18 mgm. in boring 5. The nitrates found below 6 inches were comparatively low in all of the samples, and the distribution does not seem to have been influenced greatly by the irrigation. In this grove the feeding roots were fully 6 inches below the surface, and a study of the column of averages shows that the bulk of the nitrates in the upper 15 inches of soil were located above the feeding roots.

The soil from Corona had a very high nitrate content, which varied from 1.09 to 21.88 mgm. in the upper 3 inches. It is observed that the highest nitrate content in the surface layers occurred about midway between the furrows. The lateral movement of nitrates was also apparent at a depth of 6 to 9 inches, but below that depth there seemed to be no movement of nitrates away from the furrows. This grove was given rather deep cultivation, which kept the feeding roots well below the surface 6 inches. In spite of the large accumulation of nitrates in the upper 6 inches the supply within reach of the roots would seem to have been sufficient for the needs of the trees. However, with a supply of nitrogen above the roots sufficient for the needs of the tree for a period of at least 18 months, it would seem that the loss of nitrogen from leaching or other causes may have been considerable.

The figures for the first three groves in the Lordsburg section, all of which were on very light soils, show a strong lateral movement only in soil A, in which the nitrates in the upper 3 inches varied from 0.39 to 20.27 mgm. In soils B and C there appeared to be very little lateral movement of nitrates, but there was a large accumulation of nitrates in the surface 6 inches. Because of the very light character of these soils, all the groves are cultivated deeply, and the nitrates which accumulate in the upper 6 inches of soil can be of little use to the tree during the present season. In soil C the nitric nitrogen in the upper 3 inches averaged 19.34 mgm., while the average nitrate supply within reach of the roots was only 0.27 mgm. Such a distribution in these extremely light soils must lead to very heavy losses of nitrogen from leaching. In soil D, which is a heavy clay, the highest nitrate content was found in boring 4, which was located about midway between the furrows. In this soil there were a few feeding roots within 4 inches of the surface, but even if we consider all the nitrates available except those confined in the surface 3 inches, there was approximately two-thirds of the nitric nitrogen which was unavailable.

The distribution of nitrates in soils at Orange, Anaheim, and Whittier is shown in Table XXIV. In the grove at Orange the highest nitrate content for each section was found in boring 4, which indicates that irrigation water caused a somewhat uneven lateral distribution of nitrates.

About 90 per cent of the nitrates in the surface foot of soil were located in the upper 6 inches, in which no feeding roots could be found.

TABLE XXIV.—*Distribution of nitrates at right angles to furrows in soils at Orange, Anaheim, and Whittier, Cal.*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Locality and date of sampling.	Depth.	Boring No.								Average.
		1	2	3	4	5	6	7	8	
	<i>Inches.</i>									
Orange (sampled Aug. 29, 1916).....	0-3	.....	10.02	19.71	29.58	9.14	.....	.....	.....	17.11
	3-6	1.44	1.97	3.26	7.81	1.86	0.78	.....	.....	2.85
	6-9	.81	1.06	1.02	2.00	1.02	1.09	.....	.....	1.17
	9-12	.67	.67	.46	1.20	1.13	.99	.....	.....	.85
Anaheim (sampled Aug. 29, 1916).....	0-3	.....	.85	2.98	7.99	1.16	.....	.....	.....	3.24
	3-6	.43	.43	3.19	1.72	.32	.39	.....	.....	1.08
	6-9	.60	.25	1.27	.74	.39	.39	.....	.....	.59
	9-12	.22	.29	.71	.32	.32	.04	.....	.....	.31
Whittier (sampled Aug. 30, 1916).....	0-3	.....	18.97	19.39	36.08	9.67	24.04	9.49	.....	19.61
	3-6	1.58	1.51	8.89	6.79	8.04	2.72	1.87	3.47	4.43
	6-9	1.29	1.87	2.19	3.76	3.69	2.34	1.04	.82	2.12
	9-12	3.30	3.44	6.96	5.61	3.39	1.67	.66	.32	3.17
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	4.17
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	5.15
	36-48	.....	.....	.....	.....	.....	.....	.....	.....	4.14
	48-60	.....	.....	.....	.....	.....	.....	.....	.....	5.40

The Anaheim soil, although comparatively low in nitric nitrogen, showed a very uneven distribution, which was apparently due to the lateral movement caused by the irrigation water.

The Whittier soil was unusually rich in nitric nitrogen, and, while it showed a very large accumulation at the surface, the lateral distribution was more uniform than in most furrow-irrigated soils. It would seem that several crops of fruit must be grown before the nitrates in this soil can be utilized, and in the meantime the irrigation and rainfall will probably have carried away much of the supply, causing considerable loss.

During the month of August samples were taken from groves at Redlands, Highland, and Rialto. The distribution of nitrates in these soils is shown in Table XXV. The two soils from the Redlands district show that a large percentage of the nitric nitrogen which they contain is found in the upper 3 inches of soil, and also that the lateral distribution is very uneven.

It was apparent that the available nitrogen in grove A was not sufficient for the needs of the trees, as they showed the characteristic nitrogen-starved appearance. However, if the nitrates which have accumulated in the surface 3 inches of this soil could be brought within reach of the roots, the nitrogen supply would probably be sufficient for the production of a fair crop of fruit.

The trees in grove B seemed to get sufficient nitrogen, as the foliage was in good condition and they bore a good crop of fruit. On comparing the nitrate content of soils A and B below the upper 6 inches, it is seen that the latter contains about twice as much nitric nitrogen as the former.

Notwithstanding the fact that considerable quantities of commercial nitrogen have been added during the season, the nitrate content of the

Highland soil was found to be very low. This grove bore a cover crop of *Melilotus alba* at the time the samples were taken, and it is believed that the low nitrate content is due to the growth of this crop. A number of soils on which melilotus was growing have been analyzed for nitrates from time to time during the growth of the crop, and it has been found that the growth of melilotus invariably reduces the nitrate content of the soil very materially.

TABLE XXV.—Distribution of nitrates at right angles to furrows in soils at Redlands, Highland, and Rialto, Cal.

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Locality and date of sampling.	Depth.	Boring No.								Average.
		1	2	3	4	5	6	7	8	
	<i>Inches.</i>									
Redlands, soil A (sampled Aug. 22, 1916).....	0-3	.....	0.74	4.94	14.18	4.24	4.17	1.09	.....	4.89
	3-6	0.18	.32	1.00	2.18	.88	.32	.18	0.25	.67
	6-9	.18	.11	.11	.46	.25	.08	.18	.08	.18
	9-12	.11	.11	.25	.11	.18	.15	.05	.11	.14
	12-24	.....	.....	.....	.....	.....	.....	.....	.....	.11
	24-36	.....	.....	.....	.....	.....	.....	.....	.....	.18
Redlands, soil B (sampled Aug. 22, 1916).....	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.11
	0-3	.....	1.44	8.58	4.92	9.86	3.33	.57	.....	4.79
	3-6	.39	.36	.50	2.63	.53	.39	.15	.....	.71
	6-9	.46	.32	.88	.67	.39	.25	.22	.....	.46
	9-12	.22	.65	.18	.15	.22	.15	.11	.....	.24
	12-24	.....	.19	5.50	1.16	.18	.11	.13	.....	1.21
Highland (sampled Aug. 1, 1916).....	0-3	.....	.15	3.30	.18	.11	.15	.11	.30	.56
	3-6	.15	.11	.11	.15	.08	.04	.11	.04	.10
	6-9	.11	.11	.11	.08	.11	.11	.08	.08	.10
	9-12	.03	.01	.01	.01	.08	.11	.03	.01	.04
	12-24	.....	3.61	9.84	12.92	6.62	2.91	1.02	.....	6.15
	24-36	.....	.60	.95	13.20	2.77	5.50	.53	.53	3.38
Rialto (sampled Aug. 22, 1916).....	3-6	.18	.18	.67	.53	.53	.53	.11	.29	.38
	6-9	.11	.32	.74	.60	.50	.32	.18	.18	.37
	9-12	.....	.....	.....	.....	.....	.....	.....	.....	.....

The Rialto soil was extremely sandy at the surface, and below a depth of 12 inches it was so filled with coarse gravel and rock as to make sampling almost impossible. About 92 per cent of the nitrogen in the surface foot of this soil is found in the upper 6 inches, in which no feeding roots could be located. It would therefore seem that most of the nitric nitrogen in this soil can be of no value until carried down within reach of the roots. The very gravelly nature of this soil would seem to make it readily subject to leaching, and it is probable that the nitrates which have accumulated at the surface during the irrigation season will be carried far beyond the reach of the roots during the rainy season.

#### FORMATION OF NITER SPOTS IN CITRUS SOILS

The accumulation of nitrates in surface spots in western soils was first observed by Hilgard (5), who attributed their formation to a rapid nitrification of the organic matter. Regarding the effect of rainfall on the accumulation of nitrates Hilgard wrote as follows (p. 68):

Of course it is only in arid climates that the accumulation of nitrates can usually occur; for in the region of summer rains the nitrates formed during the warm season will inevitably be washed into the subdrainage, unless restrained by absorption by the roots of vegetation.



Some years later Headden (2) called attention to the occurrence of "niter spots" in Colorado soils. The occurrence of the high nitrates he attributed to the fixation of atmospheric nitrogen by nonsymbiotic bacteria. This view has been further amplified by Headden (3, 4) and also by Sackett (9). In 1910 Stewart (11) called attention to the occurrence of nitrate salts in the country rock adjacent to the "niter spot" areas. These observations led to further studies on the nitrate content of the country rock by Stewart and Peterson (12, 13, 14). As a result of their studies these authors maintain that the "niter spots" are the direct result of the leaching of the nitrates out of the preexisting deposits in the country rock and of being locally concentrated by seepage.

The data presented above show conclusively that the nitric nitrogen in furrow-irrigated soils is carried laterally from the irrigation furrows, causing a concentration of nitrates at the point at which the irrigation water meets between the furrows. If the surface soil becomes thoroughly moistened, as it frequently does during an irrigation, very rapid evaporation will occur between the moistening of the soil and the harrowing. If the lateral movement of the irrigation water is sufficient to cause a concentration of nitrates in zones, such as shown above, it would seem that the subsequent evaporation of water from the soil in which the nitrates are highest would cause a marked concentration at the immediate surface, and thus there would be formed a "niter spot" or streak, which would occupy that portion of the soil in which the nitrates are concentrated during the irrigation. Niter streaks can be readily observed in many groves if examined at the proper time after irrigation. They have a characteristic brownish appearance which varies from a light brown to a brownish black. The color probably depends upon a number of factors, among which the following seem to be important: The amount of calcium nitrate, the moisture content, soluble organic matter, and the presence of other soluble salts. Where the nitrates are associated with large quantities of alkali salts, a brownish crust is frequently formed on drying; but when the nitrates predominate, the streak or spot is only readily seen when the surface soil contains a considerable quantity of moisture. After the soil has dried and the niter streaks are scarcely visible during the heat of the day, the color may reappear during the night if the weather is foggy. This phenomenon seems to point strongly to the deliquescent character of the calcium nitrate as an important factor in producing the brown coloration. The importance of deliquescent salts in this regard is also suggested by the action of calcium chlorid, which produces a surface color so similar to that produced by the calcium nitrate that the writer has been unable to distinguish one from the other. When the nitric nitrogen in the spot amounts to 1 per cent or more, the deliquescent character of the salt seems to attract and hold small globules of water which glisten in the sunlight, giving the small spot in which the calcium nitrate has concentrated a silvery appearance. During hot, dry



weather the globules of water soon disappear; but if the weather is cool and humid the droplets may be retained for one or two days.

The samples for analyses were taken by scooping up a thin layer of soil from the brown spots two or three days after the irrigation water was turned off, but before the surface was disturbed by harrowing. The

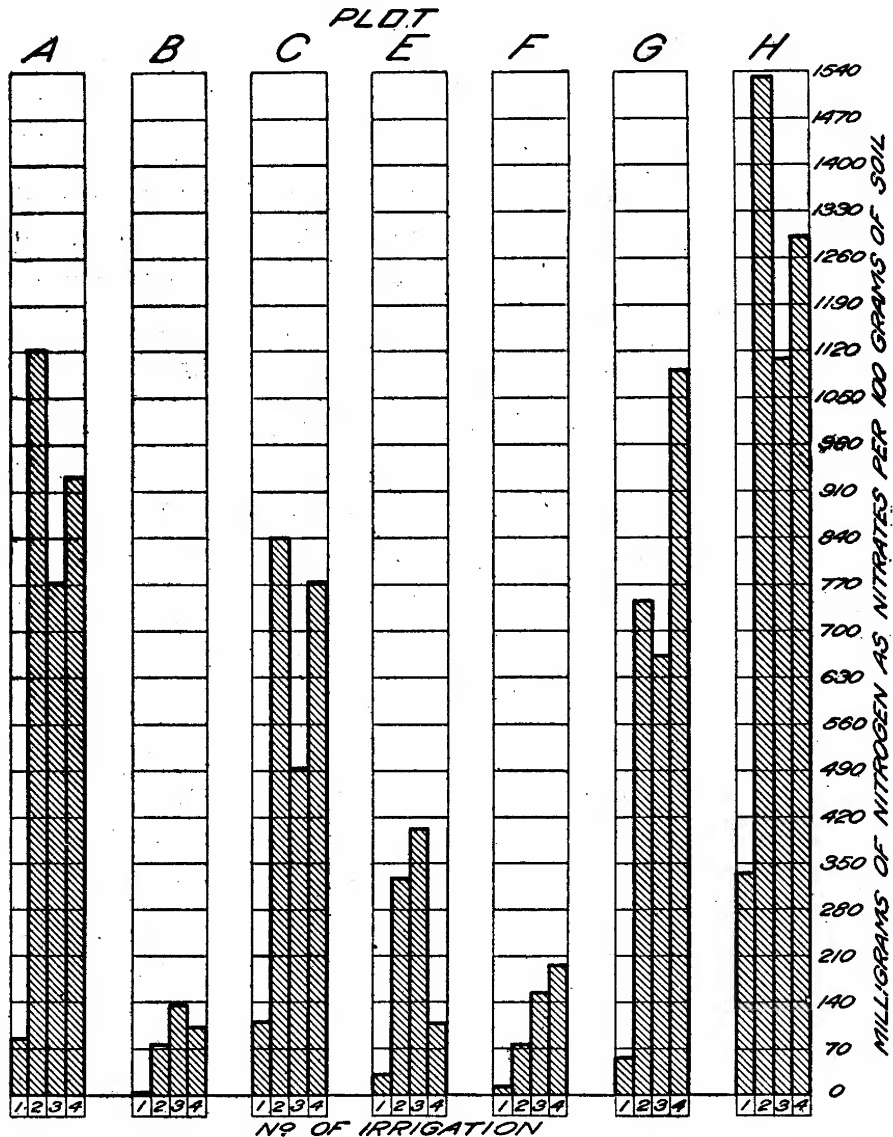


FIG. 16.—Diagram showing the nitrate content of the niter spots in furrow-irrigated soils. Season of 1916.

amount of nitric nitrogen found in the niter spots or streaks after irrigation are shown in figures 16 and 17.

The samples taken after the first irrigation show only a light accumulation of nitrates in the brown spots. At this time the brownish color characteristic of the niter spot was not abundant in any of the plots,

and in most cases the spots or streaks could be located only with difficulty. The lack of characteristic niter spots in the soil following the first irrigation is, no doubt, due to the fact that the nitrates which had accumulated at the surface during the previous season had been leached down to a considerable depth by the winter rains; and, as the new application was plowed down, there was little nitrate in the surface soil at the time of the first irrigation. The spring plowing also broke up the plowsole formed during the previous season, so that the irrigation water was not interrupted in its downward movement, and there was consequently a slower lateral movement of the water in the surface

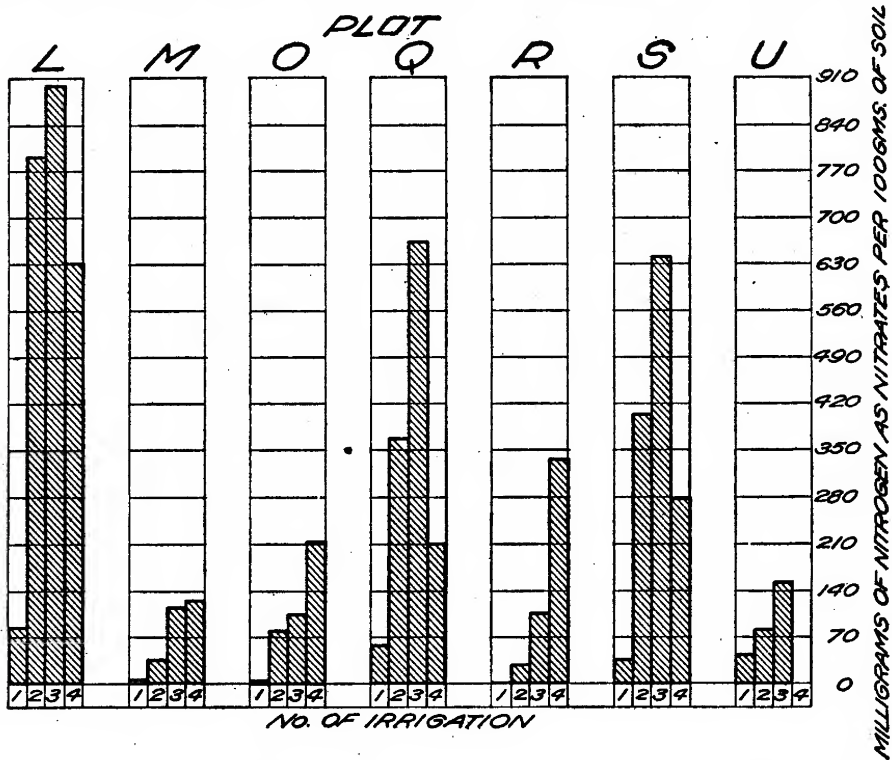


FIG. 17.—Diagram showing the nitrate content of the niter spots in furrow-irrigated soils. Season of 1916.

soil. The evaporation of moisture from the soil is also somewhat slower at this time than at the time of the later irrigations.

Following the second irrigation, niter streaks and spots were abundant in soils A, C, G, H, and L; and analyses of scrapings from these spots showed them to be very rich in nitrates. The scrapings from soil H at this time contained 1.54 per cent of nitrogen as nitrates, and soil A 1.12 per cent.

Plots B, M, and R, which have not received any nitrogenous fertilizer; plots F and O, which have received manure; plot E, which has received only a light application of nitrogen in bone meal; and plot U, which has received manure and a cover crop, show a comparatively small

amount of nitric nitrogen in the surface scrapings. It may also be stated that those plots which showed a low nitrate content in the scrapings also showed only a few niter spots, which were a light brownish color and could scarcely be distinguished after the surface soil began to dry.

After the third irrigation the nitric nitrogen found in the scrapings from the brown spots was somewhat less than after the second irrigation in plots A, C, G, and H, but was greater in all the other plots. It is seen that the nitrates in the unfertilized, lightly fertilized, and manured plots remain low as compared with those which have received large applications of nitrogen in commercial fertilizers.

After the fourth irrigation the scrapings from plots C, F, G, M, O, R, and U were higher than after any previous irrigation; but in plots E, Q, and S the amount found is much less than during the second or third irrigation. Such irregularities are to be expected, as the quantity of nitrates in the niter spots depends upon so many variable factors, such as the physical character of the soil, length of irrigation period, rapidity with which water moves laterally in the soil, rate of evaporation, length of time between irrigation and sampling, distribution of nitrates in soil at the time irrigation is started, etc.

After the consideration of only the last three irrigations it was found that the average nitrate content of the scrapings for the five plots of light soil which have received heavy applications of commercial fertilizers amounted to 0.92 per cent of nitrogen. The average nitrate content of the scrapings from the two heavy soils receiving heavy applications of commercial fertilizers was only 0.43 per cent of nitrogen. It would therefore seem that surface spots high in nitrates are less likely to be formed in heavy than in light soils, especially if the soils are underlain by a rather impervious plowsole.

The amount of nitric nitrogen found in the scrapings from plots F, O, and U is in striking contrast to the amount found in soils receiving the same or a smaller amount of nitrogen in commercial fertilizers. The average nitrate content of the scrapings from these three plots for the last three irrigations is only 0.14 per cent, which is less than one-sixth of the amount found in the light soils receiving nitrogen in commercial fertilizers.

The surface scrapings from plots B, M, and R, which have not received any nitrogen, contain an average of 0.09 per cent of nitrogen for the last three irrigations, which is but little less than the average amount found in the three plots receiving organic matter, but which is less than 10 per cent of the amount found in the scrapings from the light soils which have received 145.8 pounds of nitrogen in commercial fertilizers.

The data presented above fail to indicate that the formation of niter spots in these soils is dependent upon the processes of nitrogen fixation

or nitrification. The soils are very poor in both organic matter and total nitrogen, and the quantity of nitric nitrogen found in the control plots shows that the nitrification of the organic matter takes place very slowly. Furthermore, if the nitrogen-fixing or nitrifying bacteria were responsible for the production of the niter spots in these soils, it would seem that the niter spots in the soils receiving applications of active organic matter would be higher in nitrates than the soils to which no organic matter was applied. It is seen that this is not the case.

Inasmuch as the higher ground surrounding the experimental field is dry land and the water table is far below the zone which would make it possible for the water to move to the surface by capillary action, it would seem that the nitrate content of the soils can not be influenced by deposits of nitrates occurring in the country rock.

The formation of niter spots or streaks in Citrus soils is so definitely correlated with the fertilization and furrow system of irrigation that it would seem that there can be no doubt of the accuracy in the interpretation of the forces responsible for their formation in these soils.

#### DISTRIBUTION OF NITRATES IN SOILS IRRIGATED BY AN OVERHEAD SYSTEM OF IRRIGATION

During the season of 1915 a number of samples were drawn from a grove at Covina which is irrigated by an overhead system.

A portion of the grove received a mulch of bean straw, while another part remained unmulched. On July 14, as shown in Table XXVI, the highest nitrate content of the unmulched soil was at a depth of 6 to 18 inches, while the mulched soil showed the highest nitrate content at a depth of 18 to 30 inches. The mulch applied to this soil had presumably prevented rapid evaporation from the soil, and the water had therefore penetrated the soil to a greater depth than in the unmulched soil, thus carrying the nitrates somewhat deeper. Another set of samples was taken from this grove on August 2. In the meantime the grove had been given a light irrigation amounting to approximately one-half inch of water. In the overhead system of irrigation used in this grove the irrigation pipes are placed over every third row of trees. The middle farthest away from the pipes usually received less water than the middles on either side of the row above which the pipes are placed. The samples taken on July 14 were from the dry middles, and the nitric nitrogen in the unmulched soil, as stated above, had apparently not been carried below a depth of 18 inches. The samples taken from the wet middle on August 2 showed the highest nitrate content at a depth of 18 to 30 inches. Since the quantity of water added between the samplings was too small to effect the distribution of the nitrates, it would seem that the heavier irrigation given the soil near the irrigation pipe had caused a downward movement of the nitrates below 18 inches, while the lighter



irrigation given the middles farthest from the pipes left the nitrates in the upper 18 inches of soil. However, as only one set of samples was taken on each date the results may be due in part, or entirely, to a lack of uniformity in distribution.

TABLE XXVI.—*Distribution of nitrates in soils at Covina, Cal., irrigated by an overhead system of irrigation. Season of 1915*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Depth.	July 14.		Aug. 2.		Oct. 28.		Nov. 20.
	No mulch; dry middle.	Mulch; dry middle.	No mulch; wet middle.	Mulch; dry middle.	Mulch; dry middle.	No mulch; wet middle.	No mulch; wet middle.
<i>Inches.</i>							
0-6.....	2. 14	2. 94	1. 81	4. 69	2. 68	1. 01	0. 63
6-18.....	4. 41	2. 08	1. 15	3. 43	2. 68	. 81	. 70
18-30.....	. 21	3. 43	4. 03	1. 33	2. 68	1. 30	. 70
30-42.....	. 12	. 25	. 28	. 28	. 98	1. 16	. 91
42-54.....							. 98
54-66.....							1. 02
66-78.....							1. 54

During the latter part of the irrigation season the grove received heavier applications of water, amounting to approximately 4 inches on August 10, 5.4 inches on September 24, and 2.8 inches on October 20. A comparison of the distribution of the nitrates in the wet and the dry middle of the mulched soil on August 2 and October 28 shows that the influence of the larger application of water is clearly apparent. In the dry middle on August 2 the bulk of the nitric nitrogen was found in the upper 18 inches of soil, while it is evenly distributed in the dry middle on October 28 to a depth of 30 inches. Samples drawn from the unmulched portion of the grove on October 28 showed a nitrate content so much below the amount found on August 2 that it was thought advisable to draw samples from the deeper layers; consequently, on November 20, samples were taken to a depth of 78 inches. In these samples the highest nitrate content was found in the soil drawn from a depth of 66 to 78 inches, thus indicating that the nitrates had been carried to a depth of several feet by the irrigation water applied during the latter part of the summer.

During the season of 1916 a large number of samples were taken from the same grove at Covina on August 9, August 30, and September 2. The soil samples were taken by driving a rectangular tube (2 by 4 by 18 inches) into the soil to a depth of 3 inches, thus removing a block of soil 2 by 4 by 3 inches.

This method of sampling proved quite satisfactory when the moisture content of the soil was sufficient to cause the soil to pack into the tube so that the exact block desired could be removed. When the soil was light in character and the moisture content low, it was necessary to drive the



sampling tube to the desired depth and then drive under a thin iron plate to prevent the soil from falling out as the tube was removed.

On August 9 five successive sets of 12 samples each were drawn from a strip of soil 2 inches wide, 48 inches long, and 15 inches deep. The nitric nitrogen as determined in each sample is shown in Table XXVII. The upper 3 inches of soil showed an average nitrate content of 0.70 mgm. In the second 3-inch section the average is only 0.19 mgm., with the variation in individual samples from 0.08 to 0.25 mgm. The soil at a depth of 6 to 9 inches shows an average nitrate content of only 0.14 mgm., with a variation in individual samples from 0.05 to 0.29 mgm. The fourth and fifth sections show a higher nitrate content than the second or third.

TABLE XXVII.—*Distribution of nitrates in soils at Covina, Cal., irrigated by an overhead system of irrigation. Season of 1916*

[Results expressed as milligrams of nitrogen per 100 gm.. of soil]

Date of sampling.	Depth.	Boring No.												Average.
		1	2	3	4	5	6	7	8	9	10	11	12	
Aug. 9.....	Inches.													
	0-3	0.64	0.71	0.71	0.50	0.92	0.64	0.64	0.20	0.78	0.57	0.57	0.50	0.70
	3-6	.19	.15	.15	.22	.08	.25	.22	.15	.22	.19	.22	.22	.19
	6-9	.05	.12	.15	.12	.15	.15	.15	.12	.08	.29	.15	.15	.14
	9-12	.22	.15	.29	.29	.36	.22	.26	.29	.15	.15	.15	.15	.22
Aug. 30 (before irrigation)...	12-15	.19	.26	.22	.29	.29	.22	.29	.26	.19	.29	.29	.22	.25
	0-3	1.16	1.37	1.30	1.30	.74	.67	.88	.88	.95	1.06	.92	.88	1.01
	3-6	.36	.22	.22	.25	.39	.39	.39	.25	.29	.53	.25	.22	.31
	6-9	.32	.25	.18	.22	.22	.39	.74	.36	.36	.32	.22	.18	.31
	9-12	.15	.15	.15	.15	.25	.32	.46	.22	.39	.25	.11	.15	.23
Sept. 2 (after irrigation)...	12-24													.15
	24-36													.18
	36-48													.32
	0-3	.43	.39	.53	.60	.53	.60	.57	.46	1.02	.39			.55
	3-6	.36	1.16	.95	.46	.64	.29	.39	.46	.39	.50			.56
	6-9	.36	.29	.53	.67	.74	.95	.67	.60	.46	.46			.57
	9-12	.46	.50	.64	1.44	.95	1.13	.60	.39	.81	.57			.75
	12-24													.92
	24-36													.32
	36-48													.18
	48-60													.08

In this series of samples it is seen that the lateral distribution of the nitrates is quite uniform, but that there is some tendency for the nitrate to accumulate in the surface 3 inches even under an overhead system of irrigation.

In order to study more exactly the effect of overhead irrigation on the distribution of nitric nitrogen, two additional sets of samples were taken, one on August 30 just before the irrigation and the other on September 2 after an application of about 3 inches of water. On August 30 the average nitric-nitrogen content in the upper 3 inches was 1.01 mgm., which was more than the total amount found in the next three sections. On September 2 the highest nitrate content was found at a depth of 12 to 24 inches, while the surface 3 inches contained the smallest amount found in the upper 2 feet. The figures presented in Tables XXVI and XXVII would seem to leave little doubt that the overhead irrigation gives a much better distribution of nitric nitrogen than can be secured under the

furrow system. Under the overhead system it would seem that the nitrates which are formed or carried above the feeding roots by capillary action between irrigations may be leached down within reach of the roots with every application of water. On the other hand, the results secured during the season of 1915 indicate that much nitric nitrogen may be carried below the reach of the roots if too much water is applied.

#### DISTRIBUTION OF NITRIC NITROGEN IN BASIN-IRRIGATED SOILS

In basin irrigation the field is laid off into compartments, or checks, wholly surrounded by levees, with a tree in the center of each. The water is admitted at the upper end; and, if the basin is properly constructed, the water spreads uniformly over the bottom of the compartment, or basin. Under this system of irrigation there is less opportunity for a lateral movement of the water, such as takes place under the furrow system, in which the water is applied in small streams several feet apart.

The distribution of nitrates in basin-irrigated soils was determined by selecting a strip of soil 2 inches in width and from 40 to 48 inches in length and removing small blocks of soil having a dimension of 2 by 4 by 3 inches until all the soil was removed to a depth of 12 or 15 inches. The nitric nitrogen in each block of soil was determined separately. In three of the basins studied a large number of borings were made in the bottom of the trench in foot-sections to a depth of 48 inches, and the average nitrate content of each 12-inch section determined. The distribution of nitrates in basin-irrigated and mulched soils is shown in Table XXVIII.

TABLE XXVIII.—*Distribution of nitrates in basin-irrigated soils*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Locality and date of sampling.	Mulch.	Depth.	1	2	3	4	5	6	7	8	9	10	11	12	Average.
		<i>Inches.</i>													
Whittier, Aug. 30, 1916.	Bean straw....	0-3	1.76	1.62	2.35	2.98	3.85	3.97	3.97	4.02	4.35	4.12	.....	.....	3.30
		3-6	1.16	1.41	1.34	1.34	.88	1.37	1.65	1.72	2.00	2.35	.....	.....	1.52
		6-9	1.51	2.00	2.25	1.16	.57	.81	.74	3.33	1.23	1.62	.....	.....	1.52
		9-12	1.58	1.76	3.05	2.25	.74	.60	.99	.60	.46	.74	.....	.....	1.28
		12-24	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.71
		24-36	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.63
		36-48	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.25
		0-3	1.97	2.35	3.77	3.44	3.54	2.97	3.82	3.86	3.65	4.42	4.56	3.44	3.48
		3-6	1.02	1.06	1.41	2.00	2.35	2.77	2.25	3.16	3.26	2.49	3.25	2.07	2.26
		6-9	.67	1.23	1.58	2.00	2.42	3.12	2.49	2.46	3.72	3.40	2.67	2.81	2.38
Plot 4. Citrus Experiment Station grove, Arlington, Sept. 12, 1916.	Alfalfa hay....	9-12	1.30	1.37	1.72	1.51	2.49	3.19	2.63	2.21	3.53	3.12	3.74	2.56	2.45
		12-24	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.93
		24-36	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.55
		36-48	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.65
		0-3	1.37	.71	.88	.95	.81	1.16	.53	.50	.67	.64	.50	.60	.78
Plot 27. Citrus Experiment Station grove, Arlington, Sept. 12, 1916.	Barley straw..	3-6	.46	.25	.32	.39	.25	.25	.29	.29	.25	.32	.25	.25	.30
		6-9	.46	2.00	.25	.46	.15	.17	.16	.11	.15	.18	.11	.18	.36
		9-12	.25	.39	.15	.15	.04	.15	.11	.11	.11	.15	.11	.11	.15
		12-24	.....	.....	.....	.....	.04	.....	.....	.....	.....	.....	.....	.....	.15
		24-36	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.29
Riverside, July 27, 1916.	Alfalfa hay....	36-48	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.57
		0-3	.32	.32	.32	.32	.25	.29	.32	.39	.29	.39	.29	.32	.32
		3-6	.18	.15	.11	.11	.11	.15	.39	.18	.11	.11	.15	.11	.15
		6-9	.11	.08	.04	.08	.11	.08	.11	.11	.11	.11	.08	.15	.10
		9-12	.04	.04	.18	.11	.04	.08	.08	.11	.18	.11	.25	.11	.11
Highgrove, July 27, 1916.	...do.....	12-15	.08	.08	.11	.11	.11	.08	.08	.08	.04	.04	.08	.04	.08
		0-3	1.09	1.02	1.23	1.06	1.09	.88	1.06	.96	.53	.39	.53	.46	.86
		3-6	.60	.60	.81	.67	.53	.46	.39	.39	.32	.32	.32	.39	.48
		6-9	.46	.39	.32	.39	.39	.39	.25	.32	.25	.32	.29	.39	.35
		9-12	.46	.46	.46	.53	.50	.60	.46	.32	.32	.32	.25	.60	.44
		12-15	.39	.32	.39	.39	.32	.32	.25	.25	.25	.25	.32	.32	.32

The basin at Whittier mulched with bean straw contains an abundance of nitric nitrogen, which is less evenly distributed than might be expected under this system of irrigation. In the first two sections there is a tendency for the nitrates to increase from boring 1 to boring 10. The uneven distribution is believed to be due to the fact that the bottom of the basin was about 3 inches lower under boring 1 than under boring 10. The low spots in the basin not only receive the greatest amount of water, but are covered by the heaviest mulch. The evaporation of water from a soil takes place most rapidly from the highest points if the capillary action is not interrupted. Furthermore, the mulch is thinnest on the high ground; therefore the concentration of nitrates would naturally occur at these points. However, the distribution of the nitrates is much better than the distribution found in the adjacent furrow-irrigated soil (see Table XXIV). In the basin-irrigated soil only about 7.7 per cent of the nitrogen in the first 4 feet is found in the upper 3 inches of soil, while in the adjacent furrow-irrigated soil about 24 per cent of the nitric nitrogen in the first 4 feet is found in the upper 3 inches.

The basin at Arlington mulched with alfalfa hay shows a very satisfactory distribution of nitrates. In the upper 3 inches the variation is from 1.97 to 4.56 mgm. The second section shows a variation from 1.02 to 3.25 mgm. In the third section the variation is from 0.67 to 3.72 mgm. The next section shows a variation of from 1.30 to 3.74 mgm. The column of averages shows that the vertical distribution is quite satisfactory. As in the Whittier soil, the highest average is found at a depth of 12 to 24 inches.

The basin at Arlington mulched with barley straw contains much less nitric nitrogen than the basins discussed above, and the vertical distribution is somewhat less satisfactory. The highest amount of nitrates are found in the upper 3 inches and the second highest at a depth of 36 to 48 inches, which, for this soil, is probably below the bulk of the feeding roots.

The basin at Riverside mulched with alfalfa hay is very low in nitrates, but the distribution is fairly uniform.

The basin in the Highgrove section does not contain large amounts of nitrates; but, as the supply is very well distributed, the amount is probably sufficient for the needs of the trees.

While the studies on the distribution of nitric nitrogen in basin-irrigated soils are too limited to warrant any definite conclusion at this time, it would seem that the distribution of nitric nitrogen in basin-irrigated soils is far more satisfactory than in furrow-irrigated soils. The substitution of a mulch for cultivation also permits the feeding roots to come near the surface, and, thus, the nitrates and other plant food in the surface layer can be more completely utilized than under the furrow system.

## EFFECT OF RAINFALL ON THE DISTRIBUTION OF NITRATES IN CITRUS SOILS

The effect of rainfall on the distribution of nitric nitrogen during the season of 1914-15 is shown in figure 18. The rainfall from November 12, 1914, to January 23, 1915, amounted to 3.36 inches, but it fell in light showers over a period of nearly 10 weeks and seems to have had

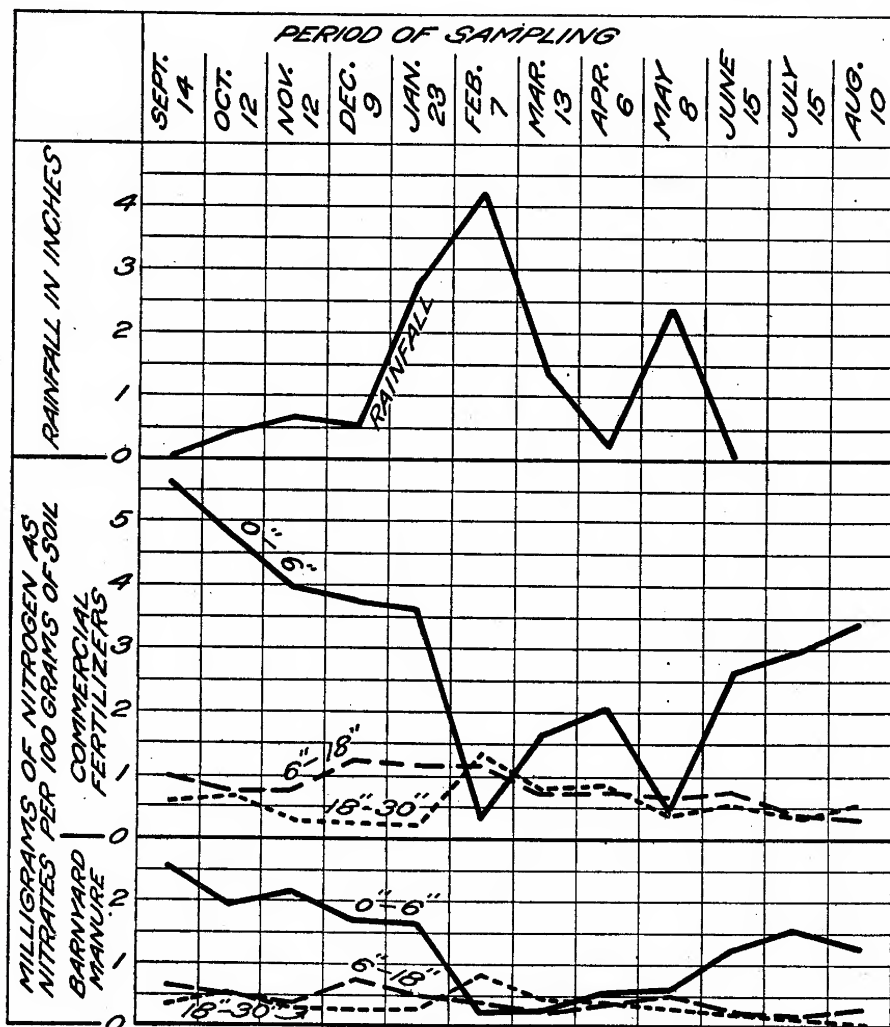


FIG. 18.—Diagram showing the effect of rainfall November 12, 1914, to July 15, 1915, on the distribution of nitrates in soils at Riverside, Cal.

little effect on the movement of nitrates in the soil. From January 23 to February 7 the rainfall amounted to 4.22 inches. The effect of the rainfall during this period on the distribution of nitrates is very marked. In the soils which had received commercial fertilizers the nitrates in the upper 6 inches fell from 3.62 to 0.31 mgm. There was no increase in nitrates at a depth of 6 to 18 inches; but at a depth of 18 to 30 inches



there was an increase from 0.23 to 1.37 mgm., indicating that a portion of the nitrates removed from the surface 6 inches was deposited at a depth of 18 to 30 inches. In the soils which have received barnyard manure the nitrates in the upper 6 inches of soil amounted to 1.65 mgm. on January 23, and on February 7 only 0.25 mgm. was found. During the same period there was an increase in nitrates at a depth of 18 to 30 inches from 0.30 to 0.85 mgm., thus confirming the results secured in the soils receiving commercial fertilizers.

There is a marked increase in nitrates in the upper 6 inches of the soils receiving commercial fertilizers from February 7 to April 6. This increase is due to the application of nitrogenous fertilizers from February 27 to March 1. The rainfall from February 27 to April 6 amounted to only 0.56 inch; and, as this quantity fell in light showers, the rainfall during this period could have had little or no influence on the distribution of the nitrates. It is interesting to note that, while there is a marked increase in nitrates in the upper 6 inches of soil of the plots receiving commercial fertilizers, there is little or no increase from 6 to 18 or from 18 to 30 inches in these soils. Very little increase is seen in the soils receiving barnyard manure, even in the upper 6 inches. As the manure was applied in February, it would seem that the nitrogen in manure becomes available very slowly in these soils.

From April 15 to 18 about 3 acre-inches of irrigation water were applied, and from April 20 to May 2 the rainfall amounted to 2.24 inches. The soil was well moistened by the irrigation; and, as the weather conditions during this period were such as to permit only slight loss from evaporation, the rainfall during this period was very effective in moving the nitric nitrogen out of the surface layers. On April 6 the average nitrate content of the upper 6 inches of the seven plots receiving commercial fertilizers amounted to 2.07 mgm. On May 8 the amount found was only 0.42 mgm., and there was also some reduction in the lower layers, indicating that the nitrates moved from the surface were carried downward to a considerable distance.

The nitrates in the manured soils were low during the spring, and the rainfall from April 20 to May 2 seems to have had little effect on the nitrates in these soils. However, it is quite possible that nitrification of the manures from April 6 to May 8 was sufficient to maintain the low nitrate content in these soils against the leaching effect of the rains.

It is observed that a very marked increase in nitrates has taken place from May 8 to June 15 in the upper 6 inches of soil in the plots receiving commercial fertilizers, but that the increase below the 6-inch layer is very slight and in some cases no increase is observed. The marked increase in the upper 6 inches of soil at this period is no doubt due to the second application of fertilizer which was added May 15.

There is also an increase in nitrates in the upper 6 inches of the manured soils, which indicates that the nitrification of the manure was now



taking place more rapidly than during the first two months after its application. There is a decrease in the nitrates from 6 to 30 inches in the

manure plots, which is possibly due to the assimilation of the nitrogen by the trees.

Six sets of samples were taken from groves at Lordsburg on January 7, 1916. A second set of samples was taken on January 26. Each sample for analysis was made up of six borings, and the borings required for each set of samples were located as near each other as practicable. Between January 7 and January 26 there was a rainfall of 14.89 inches. The effect of the rainfall is shown in figure 19.

Soil 1 contained only a small quantity of nitric nitrogen at the time the first set of samples was taken, but the amount contained was rather evenly distributed to a depth of 42 inches. On January 26 the nitrates in soil 1 had been reduced to about one-fourth of the amount present on January 7. The amount of nitrates found below a depth of 42 inches indicates that the rains between January 7 and 26 had carried the nitrates removed from the upper layers of soil below a depth of 96 inches.

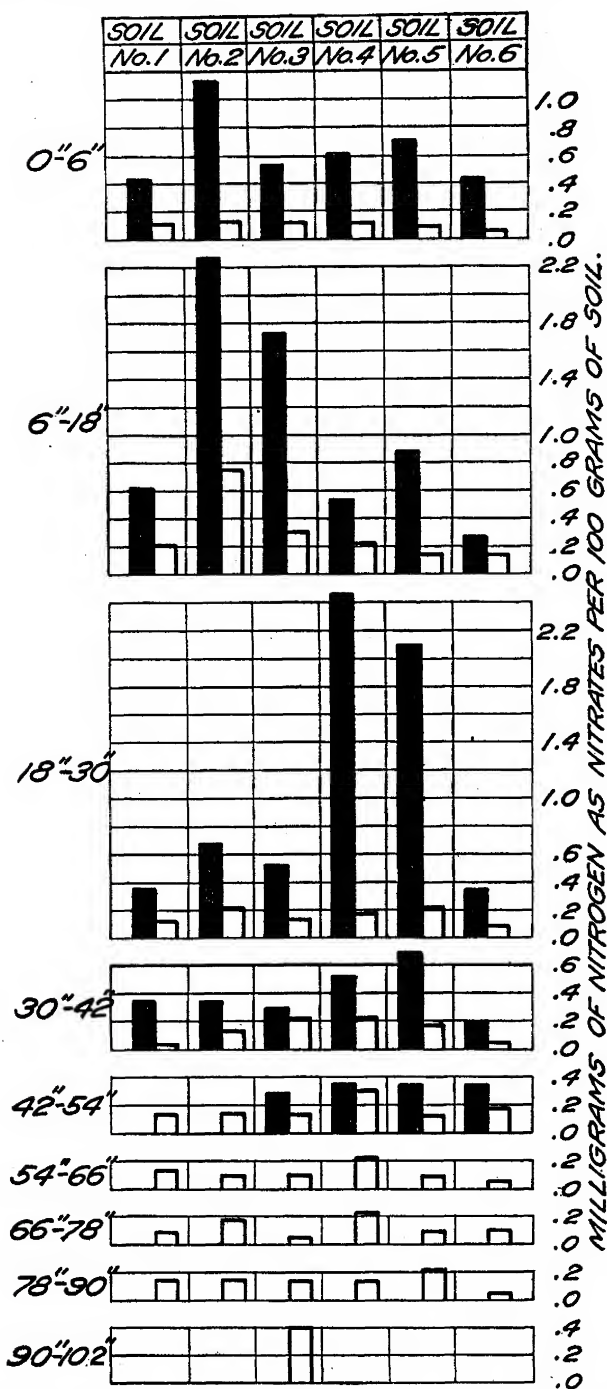


FIG. 19.—Diagram showing the effect of rainfall January 7 to January 26, 1916, on the distribution of nitrates in soils at Lordsburg, Cal. The black columns show the nitrate content of the soils on January 7 and the blank columns of January 26.

Soil 2 contained about 162 pounds of nitric nitrogen per acre on January 7 in the upper 3½ feet. After the heavy rains between January 7 and 26, the soil was found to contain only about 70 pounds to a depth of 90 inches. It would therefore seem that the loss of nitrogen in this soil from these rains was at least 100 pounds per acre.

Soil 3 is a heavy clay soil which contained a considerable quantity of nitrogen at a depth of 6 to 18 inches on January 7. About three-fourths of the nitrates contained in this soil seems to have been lost by leaching between January 7 and 26.

Soils 4 and 5 were taken from different parts of the same grove, and both were found to contain considerable quantities of nitrates on January 7. The loss in these soils must have amounted to well above 100 pounds per acre as a result of the rainfall from January 7 to 26.

Soil 6 contained only a little nitric nitrogen at the time the first set of samples was taken, but the amount found after the heavy rains is much smaller. The small amount of nitric nitrogen found from 66 to 90 inches indicated that the nitrates removed from the surface layers in these soils had been carried well beyond the reach of the roots and beyond the depth where capillary action might be a factor in returning them to the surface layers.

From September 30 to October 7, 1916, the rainfall at Riverside amounted to 1.80 inches. Immediately after these rains samples were taken from three soils at Riverside and one at Arlington. At least 75 per cent of the nitric nitrogen in the first foot of these soils was located in the upper 3 inches before the rains. The distribution of nitrates in the soils after the rains is shown in Table XXIX.

TABLE XXIX.—*Effect of rainfall from September 30 to October 6, 1916, on the distribution of nitrates in Citrus soils*

[Results expressed as milligrams of nitrogen per 100 gm. of soil]

Depth.	Plot A.							Plot H.						
	Boring No.							Boring No.						
	1	2	3	4	5	6	Aver- age.	1	2	3	4	5	6	Aver- age.
<i>Inches.</i>														
0-3.....	0.11	0.11	0.11	0.04	0.04	0.11	0.09	0.32	1.02	0.22	0.18	0.46	0.50	0.45
3-6.....	1.93	.67	1.02	1.65	1.16	2.07	1.42	15.10	7.39	1.30	1.90	4.38	13.20	7.21
6-9.....	4.87	0.55	1.93	2.35	3.19	1.72	3.43	6.20	4.03	.88	9.70	6.13	3.75	5.12
9-12.....	.60	2.00	.67	.32	.39	.11	.68	2.35	.60	.60	1.16	3.37	1.23	1.55
	Soil from Arlington, Cal.							Plot C.						
0-3.....	.42	.53	.39	.25	.39	.36	.39	.46	.39	.25	.29	.36	.25	.33
3-6.....	1.37	1.86	.53	.39	.95	.25	.89	4.66	3.02	1.79	.53	1.02	1.51	2.09
6-9.....	.53	.32	.22	.32	.39	.15	.32	4.52	4.73	2.42	2.00	1.30	1.44	2.74
9-12.....	.15	.25	.11	.11	.15	.04	.14	.67	.88	.99	.88	.60	.47	.75

Samples taken from plot A on October 7 show that the distribution of the nitrates has been materially changed by the 1.80 inches of rain. After the rain the largest amount of nitric nitrogen, as shown in Table XXIX, is found at a depth of 6 to 9 inches and the next largest at a depth of 3 to 6 inches.

The distribution of nitric nitrogen in soils C and H on October 7 also shows that the 1.8 inches between September 30 and October 7 caused a movement of the nitrates out of the upper 3 inches of soil into the second and third sections.

Samples taken from a clay soil at Arlington after the rain show the highest nitrate content at a depth of 3 to 6 inches, while the surface 3 inches contains but little more than the soil from 6 to 9 inches.

The rain between September 30 and October 7 fell on the soils just as they were being prepared for irrigation, and consequently the moisture content was low. In taking the samples for analysis it was observed that the rain had been absorbed by the upper 9 inches of soil. In studying the columns of averages in Table XXIX it is seen that the movement of nitrates was also within the upper 9 inches of soil, the nitrates being leached out of the upper 3 inches and deposited at a depth of 3 to 9 inches.

#### RELATION OF NITROGEN TO MOTTLE-LEAF

Mottle-leaf, as applied to Citrus plants, is frequently accompanied by marked reduction in quantity and quality of fruit, and in advanced stages the vigor of the tree is also much impaired. As the mottling of Citrus plants had become quite widely distributed the cause of mottling has received much attention from a number of investigators during recent years. Many causal agents have been suggested, but none of the theories advanced seems to offer an entirely satisfactory explanation of this disease.

The relation of available nitrogen to mottle-leaf has been discussed by Kellerman and Wright (6) and also by Lipman (7). The total nitrogen in a large number of Citrus groves showing various degrees of mottling has been reported upon by Briggs, Jensen, and McLane (1).

In the work reported in this paper much attention has been given to the formation and distribution of ammonia and nitrates, and their possible relation to mottling has been kept in mind. In the early part of the work it was shown that the ammonia content of the soils studied was apparently changed very little by the difference in soil treatment. The application of large quantities of nitrogenous fertilizers caused only a small increase in the ammonia, and the writer has not been able to secure any evidence that any relationship exists between the ammonia content of the soil and the character of the trees or fruit produced.

However, during the progress of the work it was observed that those plots which received larger applications of nitrogen in commercial fertilizers generally bore badly mottled trees. The trees which received no

nitrogen generally showed little mottling, as did the trees receiving barnyard manure, especially when the manure was combined with a cover crop. The data presented in figures 3 to 17 show that the highest accumulation of nitrates was found in plots A, C, G, H, and L, and also that the seasonal variation in nitrates is quite marked in these soils. A study of the degrees of mottling shows that the trees on these plots are more mottled than the trees on the other plots of the grove. In many other groves extreme mottling is frequently associated with high nitrate content, although some notable exceptions have been observed.

It is well known that the percentage of nitrogen in plant tissues varies with the available nitrogen content of the soils, and it would seem that if mottle-leaf is induced by the assimilation of excessive amounts of nitrogen an analysis of mottled leaves, if taken at the time of mottling, should show a higher nitrate content than healthy leaves of the same age from the same tree. On October 26, 1916, a quantity of healthy and mottled leaves was collected from seven individual trees on plots A, H, O, R, S, T, and V. The leaves selected were all formed during the late summer and were of as nearly the same age as it was possible to secure.

In order to obtain a representative sample of the two types of leaves, 100 gm. of clean leaves were selected from each tree. The percentage of moisture and nitrogen found in the leaves is shown in Table XXX. It is observed that the moisture content of the mottled leaves is invariably higher than in the healthy leaves. The nitrogen content of the mottled leaves is also higher except from trees in plots R and T, neither of which has received any nitrogenous fertilizer. However, it is observed that the nitrogen content of the leaves from trees on plots R and T is much below that found in leaves taken from trees on plots which have received nitrogenous fertilizers. It would therefore seem that the fertilization has increased the nitrogen content of the leaves. However, it is also seen that the healthy leaves from trees on the fertilized plots are much higher in nitrogen than the mottled leaves from the unfertilized plots.

TABLE XXX.—Moisture and nitrogen content of healthy and mottled orange leaves

Plot.	Moisture.		Nitrogen.	
	Healthy leaves.	Mottled leaves.	Healthy leaves.	Mottled leaves.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
A. ....	61. 62	66. 56	2. 69	3. 41
H. ....	60. 86	66. 37	2. 92	3. 93
O. ....	60. 58	66. 67	2. 91	3. 25
R. ....	62. 10	63. 57	1. 99	1. 99
S. ....	62. 67	68. 08	2. 89	3. 34
T. ....	60. 10	63. 71	2. 00	2. 00
V. ....	60. 86	63. 52	3. 06	3. 40

It is well known that the addition of nitrogenous fertilizers to Citrus soils may be influential in bringing about important changes in the chemical composition of the soils, and it is possible that these changes, especially in the absence of organic matter, may be responsible, in some measure at least, for the apparent correlation between high nitrate content and mottling.

It must also be recognized that high nitrate content of the surface soil is frequently associated with unfavorable soil conditions. In a large percentage of badly mottled groves a rather impervious plowsole develops just below the cultivated zone. The plowsole is a serious obstacle to irrigation and frequently results in an inadequate soil moistening and a very uneven and unsatisfactory distribution of the nitrates and other soluble plant food. It would seem that the extremely variable supply of plant food and soil moisture may be an important factor in mottling. Indeed, Smith and Smith (10), in 1911, expressed the view that the most prevalent type of mottling is due to such conditions.

#### SUMMARY

(1) Semiarid soils frequently fail to nitrify dried blood when added in 1 per cent quantities, but invariably nitrify blood when added in amounts not greater than are ordinarily applied under the field conditions.

(2) The addition of dried blood to semiarid soils in 1 per cent quantities frequently caused large amounts of ammonia to accumulate in the soil. The addition of dried blood or other nitrogenous substances applied as fertilizers caused no marked increase in the ammonia content of the soils.

(3) When 1 per cent of dried blood is added to semiarid soils, as much as 50 per cent of the nitrogen added may be lost during an incubation period of six weeks. As the soils frequently give off a strong ammoniacal odor, it is believed that this loss is due, in a large measure at least, to the volatilization of ammonia.

(4) Ammonification or nitrification studies on semiarid soils in which 1 per cent of dried blood is added are of questionable value and may lead to erroneous conclusions.

(5) Green manures, especially the legume varieties, nitrify very rapidly. As much as 50 per cent of the nitrogen contained in green plant tissues may be converted into nitrates in 30 days.

(6) Green manures furnish a valuable source of energy for the non-symbiotic nitrogen-fixing organisms.

(7) The furrow system of irrigation frequently causes a very unsatisfactory distribution of the soil nitrates. In many Citrus groves more than two-thirds of the nitric nitrogen in the upper 4 feet of soil is found in the surface 6 inches, in which, because of the frequent cultivation, few feeding roots are found.



(8) The furrow system of irrigation frequently causes the formation of niter spots. Surface scrapings from these spots in heavily fertilized groves may contain as much as 1 per cent of nitrogen as nitrates.

(9) The brown color which characterizes the niter spots is probably due to a number of factors, but it is believed that the deliquescent character of the calcium nitrate is important in this regard.

(10) Where the furrow system of irrigation is employed, the fertilizing materials should be plowed down somewhat deeper than the land is cultivated. The feeding roots will then have an opportunity to assimilate the food as it is rendered available, whereas, if it is formed within the cultivated zone, the irrigation will tend to carry it farther away from the roots.

(11) Much nitric nitrogen is lost from Citrus lands by leaching. The most effective means of preventing this loss is by growing a winter cover crop.

(12) Basin irrigation or overhead irrigation gives a more satisfactory distribution of soil nitrates than the furrow system.

(13) The basin system of irrigation seems to give greatest promise when combined with a mulching system. However, the rapidity with which organic materials rich in nitrogen decay would seem to make it inadvisable to maintain a constant mulch with these materials, as the nitrates produced will probably be far in excess of the needs of the tree, and much loss will result.

(14) Mottled orange leaves have a higher moisture content than healthy leaves of the same age from the same tree. The nitrogen content of mottled leaves is also generally higher than healthy leaves.

(15) Extreme mottling is frequently associated with a high nitrate content, but the correlation is by no means an invariable one.

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## EFFECT OF DECOMPOSING ORGANIC MATTER ON THE SOLUBILITY OF CERTAIN INORGANIC CONSTITUENTS OF THE SOIL

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### INTRODUCTION

In an investigation already reported<sup>1</sup> relative to the cause of mottle-leaf of Citrus trees in southern California, it was found that the percentage of mottling was inversely correlated with the humus content of the soil as measured by the amount of organic material extracted from the soil with a 4 per cent ammonium-hydrate solution, the calcium having previously been removed with hydrochloric acid. The examination of 120 orange groves in the Riverside, Cal., district showed a correlation of 0.67 between the mottling of the orange leaves and the reciprocal of the humus content in the soil. This association is further supported by the marked growth response of orange trees, especially on the clay-loam type in the district mentioned when irrigation water is supplied through basins mulched with organic material, the soluble organic products of the decomposition in the mulch being carried directly to the root system by the irrigation water.<sup>2</sup>

The marked growth response of Citrus trees following the addition of certain kinds of organic matter or of organic solutions derived from the decomposition of organic matter may be due to the addition of some organic or inorganic constituent, or possibly to some indirect action of the organic solution on the soil or the soil flora. The fact that chlorosis is sometimes associated with a deficiency of soluble iron, and that magnesium, according to the investigations of Willstätter<sup>3</sup> and his colleagues, is an essential constituent of chlorophyll, has made it appear desirable to determine if possible to what extent the addition of organic matter to the soil increases the solubility of these elements and other essential

<sup>1</sup> Briggs, L. J., Jensen, C. A., McLane, J. W. Mottle-leaf of Citrus trees in relation to soil conditions. *In Jour. Agr. Research*, v. 6, no. 19, p. 721-729. 1916.

<sup>2</sup> Briggs, L. J., Jensen, C. A., McLane, J. W. The mulched-basin system of irrigated Citrus culture and its bearing on the control of mottle-leaf. U. S. Dept. Agr. Bul. 499, 31 p., pl. 1. 1916.

<sup>3</sup> Willstätter, R. The chemistry of chlorophyll. *In Rpt. 79th Meeting Brit. Assoc. Adv. Sci.*, 1909, p. 667-668. 1910.

inorganic plant constituents. An economic bearing is also given to the investigation by the fact that the mulched-basin system has in some cases had a marked effect in reducing mottle-leaf on Citrus trees.

The present paper deals with the solvent action on certain inorganic soil constituents of the water-soluble decomposition products of manures and other organic fertilizers. Soils were extracted (a) with soluble organic matter obtained from decomposing green manures and from stable manures; (b) with soluble organic matter obtained from thoroughly decomposed green manures; (c) with artificially prepared humus solutions obtained by hydrolyzing organic substances with acids; and (d) with osmosed organic solutions derived from the decomposition of organic matter. In addition, stable and green manures were added directly to the soil and the effect of this treatment was noted (a) on the amounts of certain soil minerals dissolved out with water, and (b) on the change produced in the specific electrical conductivity of the soil.

#### METHOD USED IN DECOMPOSING ORGANIC SUBSTANCES

Green barley hay, sweet clover, and alfalfa were dried and chopped into small pieces, and 70 gm. of each of these substances were placed in separate large bottles, moistened to saturation with distilled water, and allowed to ferment. An equal amount of dry cow manure was similarly treated. Fourteen days later these four substances were shaken up thoroughly with 1,500 c. c. of distilled water each and the coarser material filtered out through muslin. This filtrate was then passed through a Chamberland porous filter and collected. The solid organic matter from each of the four substances was returned to its respective bottle, kept saturated, and at a later period was again shaken with distilled water as above, filtered, and the filtrate again collected. The organic solutions thus obtained were used for soil extraction.

TABLE I.—*Intervals between successive extractions of green manures*

No. of extract.	Date extracted.	Number of days since placing in bottles.	Number of days since preceding extraction.
1.....	Feb. 2	14	.....
2.....	Feb. 24	36	22
3.....	Mar. 15	55	19
4.....	Apr. 25	96	41

These organic extracts were entirely free from suspended matter. The intervals between extractions are given in Table I. The organic solutions, or solvents,<sup>1</sup> were added to the soils under investigation in the proportion of 250 gm. of soil to 500 c. c. of solvent.

<sup>1</sup> In order to avoid confusion in the mind of the reader, the term "solvent" will be used in speaking of the organic extracts obtained from the various organic substances, and which are used in making soil extracts. Relative to the soil, they are solvents, though in themselves they are systems of water-solvent and organic and inorganic solutes.

## METHOD OF ANALYSIS

The soil extracts thus obtained contained large amounts of organic matter in solution, which it was necessary to remove before proceeding with the analysis. A number of methods were tested with solutions containing known amounts of the mineral elements under investigation, together with large amounts of organic extracts known to be free from these mineral elements. Of these methods, the following gave the best results:

The soil extracts obtained with the organic solvents were made and kept slightly ammoniacal, an excess of ammonium oxalate was added, and the extracts were evaporated to dryness. The residue was ignited just sufficiently to burn off the organic matter. The ignited mass was taken up with a measured<sup>1</sup> amount of nitro-hydrochloric acid, diluted somewhat with distilled water, and heated on the water bath until everything except the free silica had gone into solution. This acid solution was made up to volume and analyzed.

The phosphoric acid was determined volumetrically by Pemberton's molybdic method. The calcium was determined volumetrically by titrating the oxalate with potassium permanganate. The magnesium was determined volumetrically by the method of Meade. The detailed methods of determining the above-mentioned three elements are substantially those given by Sutton.<sup>2</sup> The iron was determined colorimetrically by comparing the red color developed on the addition of potassium thiocyanate to the unknown solution with a standard iron solution. The color in the standard solution was developed at the same time that the color in the unknown solutions was developed, and the readings were made at once.

## SOLUBILITY OF SOIL MINERALS IN EXTRACTS OF DECOMPOSING ORGANIC MATERIAL IN DIFFERENT STAGES OF DECOMPOSITION

In the mulched basin under field conditions the products of decomposition are gradually leached into the soil. To approximate this action, the organic solvents were prepared by extracting the same samples of decomposing organic matter at intervals as shown in Table I. The amount of calcium, magnesium, phosphoric acid, and iron removed from two soils by such organic solutions prepared from cow manure, barley hay, alfalfa hay, and sweet-clover hay in various stages of decomposition is given in Table III.

To determine the solvent action of these organic solvents in excess of that of pure water alone, a number of determinations were made of the solubility of the iron, calcium, magnesium, and phosphoric acid in the

<sup>1</sup> It was necessary to use a measured amount of acid in taking up the ignited residue after evaporating the organic soil extracts to dryness, owing to the fact that the red solution formed by the addition of potassium thiocyanate to ferric iron is rendered colorless by an excess of hydrochloric or nitric acid. In working with a few parts per million of iron in the solution, it was found unsafe to have present in 100 c. c. of the solution under investigation more than 3 c. c. of concentrated nitric or hydrochloric acid, or the same amount of a mixture of both acids.

Sutton, Francis, *A Systematic Handbook of Volumetric Analysis* . . . ed. 10, 621 p., 121 fig. 1911.



soils under examination, using distilled water only as a solvent. The means of these determinations are given in Table II.

TABLE II.—Average amounts of minerals removed by distilled water from the soils used  
[Results expressed as parts per million of dry soil]

	Clay loam.				Sandy loam.			
	Iron.	Cal- cium.	Magne- sium.	Phosphoric acid.	Iron.	Cal- cium.	Magne- sium.	Phosphoric acid.
Average amounts re- moved.....	0.47	43	12	24	0.36	39	7	14
Number of determina- tions in average.....	13	13	11	11	13	10	10	10

These mean values have been deducted in each instance from the solubility of the corresponding element in the presence of the organic solvent.

The organic solvents themselves all contained calcium, magnesium, iron, and phosphoric acid in solution, so that it was necessary to determine the amount of each of these elements added to the soil along with the organic matter. These determinations are given in the first part of Table III, all results being expressed in parts per million of the weight of the dry soil used. The second part of Table III shows the concentration of each element in the organic solution after it had been shaken with the soil and freed from suspended material by filtration through a Chamberland tube. In each instance, however, the amount of each element dissolved in distilled water, as given in Table II, has been deducted.

TABLE III.—Minerals removed from soils by extracts of organic substances during various stages of decomposition

[Results expressed in parts per million of dry soil. Amounts removed by distilled water have been deducted]

Organic substances and their successive extraction.	Inorganic substances added to soil with organic extracts.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Cal- cium.	Magne- sium.	Phos- phoric acid.	Iron.	Cal- cium.	Magne- sium.	Phos- phoric acid.	Iron.	Cal- cium.	Magne- sium.	Phos- phoric acid.
Barley:												
First.....	7.00	13	76	138	0.21	508	105	51	1.69	529	66	93
Second.....	6.00	106	38	69	4.90	358	89	105	3.96	368	49	81
Third.....	2.40	58	20	26	.84	232	57	14	2.04	225	41	21
Fourth.....	.75	55	6	5	.08	78	5	—2	.09	94	5	11
Sweet clover:												
First.....	14.00	59	122	280	3.34	402	129	184	4.80	261	103	196
Second.....	23.30	125	40	60	1.00	473	132	53	1.46	512	92	77
Third.....	8.40	149	20	24	—0.02	185	52	2	.19	217	32	11
Fourth.....	1.10	86	5	10	—0.25	91	12	9	.89	99	7	13
Alfalfa:												
First.....	2.25	211	150	184	.66	472	157	88	.68	426	75	159
Second.....	.75	264	59	66	.50	523	131	71	.96	480	70	72
Third.....	.50	79	16	23	.03	195	58	22	.61	166	21	21
Fourth.....	.30	95	10	7	—0.42	133	13	13	—0.11	122	3	14
Cow manure:												
First.....	.84	91	82	135	1.46	292	92	49	1.41	211	135	103
Second.....	1.17	159	78	153	.45	216	73	96	.13	185	51	125
Third.....	.91	74	36	115	.03	84	30	43	.27	99	21	72
Fourth.....	.26	114	8	67	—0.08	83	10	25	.05	105	0	43

The various organic solvents are seen in Table III to exert a marked solvent action on the calcium and magnesium in soils. This action diminishes in intensity with the successive extracts, especially after the second. The mineral content of the organic solvents also decreases in the successive extracts, and these inorganic components doubtless play some part in the solubility of the soil minerals which can not be differentiated at this stage from the solvent action of the organic compounds.

The total amount of the inorganic elements dissolved from each soil by the four extractions, as well as the total amount of the same elements added to the soils with the organic solvents, is given in Table IV. It will be seen that the total amount of phosphoric acid and of iron recovered from the soil extracts did not equal the amount present in the organic solvents, and so added to the soil in making the extract of the latter. But the amount of these two elements dissolved from the soil was greatly in excess of the amount made soluble by water alone. In all cases the amount of calcium dissolved from the soil was much more than that added in the organic solvent, and in most cases also more magnesium was recovered than was added in the organic solvent.

TABLE IV.—*Total amounts of minerals removed from soils by the four successive organic extracts of freshly decomposing organic matter*

[Results expressed in parts per million of dry soils. Amounts removed by distilled water have been deducted]

Organic substance.	Inorganic substances added to soil with the organic extracts.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Barley .....	16.15	232	140	238	6.03	1,176	256	168	7.77	1,206	161	206
Sweet clover .....	46.80	419	187	374	4.07	1,151	325	248	7.44	1,089	234	297
Alfalfa .....	3.80	649	230	280	.77	1,323	202	194	2.14	1,194	109	366
Cow manure .....	3.18	438	204	470	1.86	675	205	213	1.86	600	207	343

It is evident from Table IV that the organic solvents obtained from the decomposing green manure were more effective in removing calcium than the organic solution obtained from the cow manure. Walters<sup>1</sup> has shown that acetic acid and proprionic acid are formed in considerable quantities through the decomposition of green rye or green alfalfa, and the greater solvent action of the solutions of green manures is perhaps attributable in part to the presence of such acids. The amount of calcium or magnesium added to the soil with the organic solvents bears no relation to the amount of these elements recovered in the soil extracts. The amount of phosphoric acid recovered varies directly with the amount added to the soil in the organic solvent. The various organic solvents

<sup>1</sup> Walters, E. H. The presence and origin of volatile fatty acids in soils. (Abstract.) *In Science*, n. s. v. 44, no. 1128, p. 217. 1916.

obtained from decomposing green manures, whether derived from a non-leguminous or a leguminous crop, showed but slight differences in their solvent action upon the elements under examination.

The total quantity of soil treated with the organic solvents would, in the case of the four materials studied, correspond approximately to a ratio of 2 parts of dry organic material to 100 parts of dry soil. The organic material was by no means completely decomposed at the time the last extraction was made. The amount of these elements dissolved from the soil as shown in Table IV was obtained during the 96 days the decomposition was in progress. We may therefore look upon the results in Table IV as representing roughly the gain in the soluble iron, calcium, magnesium, and phosphoric acid resulting from the addition of 2 per cent of organic matter to the soil, the amount dissolved from the soil by distilled water alone having been deducted. These figures show that the addition of organic matter to the soil markedly increased the amount of soluble iron, calcium, magnesium, and phosphoric acid. Expressed in pounds per acre-foot, the amounts range as follows:

Iron.....	pounds..	3 to	31
Calcium.....	do....	2,400 to	5,300
Magnesium.....	do....	640 to	1,300
Phosphoric acid.....	do....	670 to	1,400

These figures do not necessarily represent an increased solubility of the soil components, however, except in the case of calcium and magnesium. The amount of iron or phosphoric acid recovered from the soil was in no instance equal to the amount added to the soil in the organic solvent.

Separate solubility experiments were made by adding to the untreated soils the same amount of the elements under investigation as were added to the soils with the organic solvents. The salts, used individually, were the sulphates of iron, calcium, and magnesium, and dibasic sodium phosphate. Practically all the iron and about two-thirds of the magnesium and phosphoric acid added remained in the soil. On the other hand, the addition of calcium sulphate increased the solubility of calcium and magnesium, and the addition of magnesium sulphate increased the solubility of calcium.

From the results obtained it was impossible to differentiate between the solvent action of the organic and inorganic compounds in the organic solvents.

#### REACTION OF ORGANIC EXTRACTS TO INDICATORS

The organic solvents obtained from the decomposing green manures used in making soil extracts were examined as to their reaction. None of the organic solvents obtained from the first extraction after 14 days' decomposition showed an alkaline reaction with phenolphthalein or an acid reaction with methyl orange, and only occasionally was a red color

developed by boiling the extract in the presence of phenolphthalein. However, it required from 0.8 to 2.8 c. c. of normal hydrochloric acid per 100 c. c. of extract to develop an acid reaction in the extracts, using methyl orange as an indicator. Table V shows the results of the titrations of the first set of organic solvents both before and after passing through the soils.

TABLE V.—*Titration of solutions of organic substances before and after extracting soils*

Decomposing substance from which organic solution was obtained.	Normal hydrochloric acid required to develop an acid reaction in 100 c. c. of solution, using methyl orange as an indicator.		
	Organic solution.	After extracting.	
		Clay loam.	Sandy loam.
	C. c.	C. c.	C. c.
Barley hay. ....	0.80	1.00	1.00
Sweet-clover hay. ....	2.80	2.60	2.40
Alfalfa hay. ....	2.40	1.90	2.60
Cow manure. ....	1.70	1.70	1.70

There were substances, probably of organic nature, present in both the organic solvents and the soil extracts obtained with these solvents, that were neither alkaline to phenolphthalein nor acid to methyl orange. These substances, however, were capable of combining with a considerable amount of hydrochloric acid before an acid reaction was shown, using methyl orange as indicator. The amount of acid thus required varied approximately with the amount of calcium present in the solvents and extracts. This latter fact, however, does not account entirely for the comparatively large amount of acid required to make these solutions acid. Solutions from hydrolyzed organic substances containing none of the elements under investigation required about the same amount of acid to develop an acid reaction, using methyl orange as an indicator.

#### ARTIFICIALLY PREPARED MANURES

During the summer of 1915 various green-manure substances, including barley hay, sweet clover, bean straw, and alfalfa, were dried, placed in 4-gallon glazed jars, saturated with water, and allowed to ferment. At the same time a jar was filled with dry cow manure and similarly treated. Dried hay, leaves, weeds, etc., soon break down under these conditions, and in a surprisingly short time decompose into a mass closely resembling manure. Six months after these substances had been placed in the jars decomposition had evidently gone to an end, except for the slow changes which ordinarily follow the first rapid bacterial action in any manure kept in tanks.

Organic solvents obtained from these thoroughly decomposed substances were made by shaking up an equivalent of 33 gm. of dry material in 500 c. c. of distilled water, and filtering through Chamberland filters. These organic solvents were then used for making soil extracts in the proportion of 500 c. c. of the solvent to 250 gm. of soil. These soil extracts were filtered through Chamberland filters and analyzed (Table VI). The results given in Table VI show that in most instances the organic solvents obtained from the completely decomposed green manures were not as active in breaking up the soil minerals as the organic solvents from the same materials in the first stages of decomposition (see Table III).

TABLE VI.—*Soil minerals removed by extracting soils with organic solvents from completely decomposed artificial manures*

[Results expressed as parts per million of dry soil. Amounts removed by distilled water have been deducted]

Organic substance.	Added to soil with organic extracts.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Cal-cium.	Magne-sium.	Phos-phoric acid.	Iron.	Cal-cium.	Magne-sium.	Phos-phoric acid.	Iron.	Cal-cium.	Magne-sium.	Phos-phoric acid.
Barley hay.....	0.25	104	94	81	0.65	305	92	43	1.69	205	68	91
Sweet-clover hay.....	3.70	116	140	105	1.89	472	196	77	3.32	292	146	79
Alfalfa hay.....	3.20	71	88	110	1.62	411	149	30	1.41	246	93	77
Cow manure.....	.18	89	120	98	.18	290	116	83	.09	221	97	64
Bean straw.....	.98	834	247	82	.05	758	385	31	1.83	670	315	55

The organic extracts of the completely decomposed green manures were neither acid to methyl orange nor alkaline to phenolphthalein, but required a considerable amount of acid to develop an acid reaction, with methyl orange as an indicator (Table VII).

TABLE VII.—*Quantity (in cubic centimeters) of normal hydrochloric acid required to develop an acid reaction in 100 c. c. of extract from completely decomposed green manures before and after extracting soils, using methyl orange as an indicator*

Source of extract.	Organic extract.	After extracting.	
		Clay-loam soil.	Sandy-loam soil.
Barley manure.....	C. c. 2.0	C. c. (a)	C. c. 1.6
Sweet-clover manure.....	(a)	Trace.	Trace.
Alfalfa manure.....	3.8	3.2	3.3
Cow manure.....	1.6	1.6	1.6
Bean-straw manure.....	5.8	4.8	5.1

<sup>a</sup> Not determined.



## ORGANIC LIQUID FROM COMPOSTED ALFALFA

In the summer of 1915 a bale of alfalfa was placed in a galvanized tank fitted with a cover, moistened, and allowed to decompose. Water was occasionally added, and a quantity of liquid accumulated in the tank which possessed the very dark color and ammoniacal odor typical of a barnyard compost heap from which the liquid material does not escape.

This liquid was used, after some dilution, for making soil extracts. To a second portion of the liquid about 50 per cent, by volume, of alcohol was added, which precipitated part of the organic matter. This was filtered, and the filtrate and precipitate were used separately for making soil extracts, the alcohol first being driven off. The precipitate was fairly soluble in water.

A third portion of the original liquid was dialyzed through rather close-textured parchment tubing, the dialysate being frequently removed and replaced with distilled water. Both the dialysate and the organic residue were employed in making soil extracts. The organic residue in this case formed a colloidal suspension rather than a true solution in distilled water. The results of the analyses of the soil extracts made with these organic separations are presented in Table VIII.

The concentration of these various organic solvents with respect to the original alfalfa liquid was the same in all cases.

TABLE VIII.—*Soil minerals removed by extracting soils with the liquid resulting from decomposing alfalfa*

[Results expressed as parts per million of dry soil. Amounts removed by distilled water have been deducted]

Organic substance.	Inorganic substances added to soil with organic solvents.				Clay-loam soil.				Sandy-loam soil.			
	Iron.	Cal-cium.	Mag-ne-sium.	Phos-phoric acid.	Iron.	Cal-cium.	Mag-ne-sium.	Phos-phoric acid.	Iron.	Cal-cium.	Mag-ne-sium.	Phos-phoric acid.
Untreated liquid <sup>a</sup> .....	1.78	30	30	57	0.72	389	103	18	0.26	209	43	18
Alcohol soluble <sup>a</sup> .....	3.45	30	20	50	.28	645	167	10	.62	260	43	18
Alcohol insoluble <sup>a</sup> .....	2.20	32	15	64	.46	164	54	4	.26	96	19	6
Dialysate.....	.65	30	23	26	.98	516	136	4	1.78	294	47	10
Dialyzed residue.....	4.36	19	12	54	.88	25	11	15	1.45	6	—3	10

<sup>a</sup> Average of two separate determinations with two separate samples of the solvents.

All these solvents, excepting the dialyzed residue, removed considerable amounts of calcium from the soils. The magnesium was quite freely removed from the heavy soil, but less so from the light soil. Phosphoric acid was not recovered in amount equal to that added to the soil with the organic solvents, but an increase over the amount removed by distilled water was obtained. The alcohol-insoluble organic material was not as

effective in dissolving the minerals as the alcohol-soluble portion, and the organic residue from the dialyzer was the least effective. As stated above, this material when taken up with distilled water was chiefly in a state of colloidal suspension, and a strong solvent action could hardly be expected.

The amount of inorganic elements added to the soils with these different organic solvents was about the same in all cases. It would therefore appear that the solvent action of these different organic solvents was due largely to the effect of the different organic constituents present in the solvents.

The untreated organic liquid was strongly ammoniacal, requiring 4.2 c. c. of normal hydrochloric acid per 100 c. c. of solution to dispel the red color of phenolphthalein at room temperature.

#### HYDROLYZED ORGANIC MATTER

It is recognized that in the preceding experiments the inorganic salts present in the organic solvents may have exerted a solvent action on the soil minerals. In order to eliminate this effect, soluble organic extracts were prepared free from the inorganic elements under investigation.

Dried alfalfa, sweet clover, barley hay, and sugar were digested for about 24 hours on the water bath with hydrochloric acid of about 1.115 specific gravity. The acid was then washed out, the residue extracted with 4 per cent ammonia, and the solid insoluble material filtered out. The ammonia extract was then heated on the water bath, the water lost by evaporation being occasionally partly replaced, until all free ammonia was driven off. The solutions were found by analysis to be free from iron, calcium, magnesium, and phosphoric acid. They were then standardized gravimetrically. In addition to the above solutions, an organic solution was also prepared from horse manure. It was washed thoroughly with hydrochloric acid until no more calcium came through the filter. The acid was then washed out and the residue extracted with ammonia. The ammonia was driven off and the resulting organic solution standardized and used in soil extraction.

It will be seen from Table IX that the mineral-free organic solvents here used were quite effective as solvents. The sugar humus was most effective in dissolving magnesium and calcium. It also increased the solubility of the iron slightly. Its effect on the solubility of the phosphoric acid in the soils was negligible. Some of the other solvents, however, exerted a marked solvent action on the phosphoric acid in the soil.

The results obtained with the mineral-free humus solutions show clearly a solvent action on the iron, calcium, magnesium, and phosphoric acid in the soil.

TABLE IX.—Amounts of soil minerals removed from soils by solutions of artificial humus or hydrolyzed organic substances

[Results expressed as parts per million of dry soil. Amounts removed by distilled water have been deducted]

Source of humus.	Or- ganic mat- ter in sol- vent.	Clay-loam soil.				Sandy-loam soil.			
		Iron.	Cal- cium.	Magne- sium.	Phos- phoric acid.	Iron.	Cal- cium.	Magne- sium.	Phos- phoric acid.
	<i>Per ct.</i>								
Barley hay .....	0.005					0.05	0		
	.02					.19	20		
	.04					.22	38		
	.10	—0.08	108	33	21	11.64	98	2	22
Sweet-clover hay .....	.01	.06	8	9	—8	.22	9	0	3
	.03	.06	25	20	2	.14	26	5	2
	.005	.02	12			— .09	2		
	.02	.21	35			— .08	12		
Alfalfa hay .....	.04	.28	65			— .11	22		
	.01	.25	—4			.16	1	2	12
	.03	.87	34			.09	18	6	8
	.10	— .08	94	33	5	.39	66	5	9
Sugar .....	.01	.20	56	16	0	.22	160		
	.03	.29	150	49	—2	.80	242		
	.005					.09	10		
Horse manure .....	.02					.23	45		
	.04					.48	53		
	.10	1.72	115	19	53	8.64	48	3	81

Like the organic extracts of the freshly decomposing green manures, these hydrolyzed humus solutions were neutral to phenolphthalein and methyl orange. They required, however, about the same amount of hydrochloric acid as did the above-mentioned organic extracts, in order to show an acid reaction with methyl orange.

#### SOLVENT ACTION IN SOILS OF DECOMPOSING GREEN MANURES

In the study of the humification of organic substances in soils in pots, several kinds of green-manure substances were used, and to some of these pots certain inorganic salts were also added. These soils were kept moist and stirred occasionally. Samples were extracted with distilled water and the extracts analyzed for the mineral elements discussed in the preceding work. Table X shows the results obtained in one of these experiments after the organic matter had been in contact with the soil for six months. The alfalfa was slightly more effective in bringing the soil minerals into solution than the manure.

Table XI shows the results from a similar experiment with lighter soils, in which the organic matter had been in contact with the soil for three months. In this case the sweet clover did not exert as pronounced a solvent action as the manure. An increasing solvent action is shown with an increasing amount of organic matter.

TABLE X.—*Solubility of soil minerals as effected by the addition of organic manures, lime, gypsum, and sodium carbonate six months after the addition of substances to the soil*

[Results expressed as parts per million of dry soil]

Soil treatment.	Iron.	Calcium.	Magnesium.	Phosphoric acid.
Control (nothing added).....	0. 75	25	7	16
3 per cent horse manure only.....	. 39	42	7	38
3 per cent horse manure plus 3 per cent calcium carbonate.....	. 30	46	8	32
3 per cent horse manure plus 0.2 per cent sodium carbonate.....	. 51	33	9	38
3 per cent horse manure plus 3 per cent calcium sulphate.....	Trace.	1, 070	81	25
Control (nothing added).....	. 51	23	5	17
3 per cent alfalfa only.....	. 39	53	12	25
3 per cent alfalfa plus 3 per cent calcium carbonate.....	. 60	48	7	33
3 per cent alfalfa plus 0.2 per cent sodium carbonate.....	. 69	39	7	30
3 per cent alfalfa plus 3 per cent calcium sulphate.....	3. 00	925	63	18

TABLE XI.—*Solubility of iron and calcium in soils as effected by the addition of organic substances three months after the addition of substances to the soil*

[Results expressed as parts per million of dry soil]

Soil treatment.	Soil No. 1.		Soil No. 2.	
	Iron.	Calcium.	Iron.	Calcium.
Control (nothing added).....	0. 50	44	0. 80	21
Cow manure, 0.2 per cent.....	. 65	49	. 80	15
Cow manure, 1 per cent.....	. 90	53	. 90	34
Cow manure, 3 per cent.....	2. 50	58	1. 57	41
Sweet-clover hay, 1 per cent.....	. 65	29	. 77	21
Sweet-clover hay, 3 per cent.....	. 77	40	1. 53	35

In another experiment two types of soil from two orange groves near Riverside, Cal., were treated with different kinds of organic matter in different amounts, with and without the addition of certain inorganic substances. These soils were put into nonsoluble containers, placed in a greenhouse in Washington, D. C., kept moist, and stirred occasionally for a period of about 6 months. The soils were then sampled and the specific electrical conductivities determined. The results are shown in Tables XII and XIII. The specific-conductivity figures have all been multiplied by  $10^5$ .

The presence of organic matter considerably increased the amount of electrolytes in both soils. Three per cent of organic matter increased the conductivity more than did 1 per cent, and alfalfa produced a greater

solvent action than did the same quantities of barley or manure. Barley and manure in the same amounts had about equal effect in increasing the conductivity of the soils to which they were added.

TABLE XII.—*Effect of the addition of organic matter and inorganic minerals on the specific conductivity of sandy loam soil kept moist in the greenhouse for six months<sup>a</sup>*

Inorganic minerals.	Amount and kind of organic matter added.						
	None.	Manure.		Barley.		Alfalfa.	
		1 per cent.	3 per cent.	1 per cent.	3 per cent.	1 per cent.	3 per cent.
None.....	48	68	93	60	100	123	150
1 per cent of calcium carbonate.....	48	.....	100	.....	125	.....	203
3 per cent of calcium carbonate.....	55	.....	115	.....	118	.....	156
0.2 per cent of sodium nitrate.....	165	.....	207	.....	163	.....	183
3 per cent of calcium sulphate.....	73	.....	143	.....	125	.....	210

<sup>a</sup> Conductivity figures have been multiplied by  $10^5$ .

TABLE XIII.—*Effect of the addition of organic matter and inorganic minerals on the specific conductivity of clay-loam soil kept moist in the greenhouse for six months<sup>a</sup>*

Inorganic minerals.	Amount and kind of organic matter added.						
	None.	Manure.		Barley.		Alfalfa.	
		1 per cent.	3 per cent.	1 per cent.	3 per cent.	1 per cent.	3 per cent.
None.....	83	88	143	115	155	165	250
1 per cent of calcium carbonate.....	75	.....	155	.....	135	.....	230
3 per cent of calcium carbonate.....	88	.....	113	.....	138	.....	238
0.2 per cent of sodium nitrate.....	185	.....	223	.....	215	.....	325
3 per cent of calcium sulphate.....	120	.....	135	.....	175	.....	288

<sup>a</sup> Conductivity figures have been multiplied by  $10^5$ .

The addition of calcium carbonate to the heavy soil treated with organic matter caused a slight decrease in the amount of soluble salts liberated as compared with the effect of the organic matter alone. In the light soil treated with organic matter the addition of lime increased slightly the amount of soluble salts. On the whole, however, the presence of calcium carbonate did not greatly modify the formation of soluble salts in the soil.

When gypsum was added to the soil with the organic matter, the amount of soluble salts was increased beyond the increase produced when the same amount of calcium carbonate was added. This, however, is chiefly due to the greater solubility of gypsum. The solubility of gypsum is about 2 parts in 1,000, while that of calcium carbonate is much less. This is brought out also in Tables XII and XIII, which show that when 3



per cent of lime alone is added to the soil the conductivity is but slightly increased. The same amount of gypsum under the same conditions increases the conductivity about one-third.

The net effect of the organic matter in increasing the conductivity of the soil when calcium carbonate was added was about the same as when gypsum was added. This is seen by deducting the conductivities of the soils to which calcium carbonate alone was added from the conductivities of the soils to which both organic matter and calcium carbonate were added. Making similar subtraction of conductivities of the soils treated with gypsum, with and without organic matter, gives about equal conductivity results.

The addition of sodium nitrate to soils increased the conductivity from 2.5 to 3.5 times over the conductivities of soils receiving no treatments. However, when the conductivities of the soils receiving this salt alone are deducted from the conductivities of the soils receiving both organic matter and sodium nitrate it is seen that the solvent action of the organic substances in the presence of this salt is less than when the organic substances alone are present.

It is seen that the solvent action of 3 per cent of alfalfa mixed with the sandy-loam soil is almost as great as when the same soil is mixed with 0.2 per cent of sodium nitrate alone. The solvent action of 1 per cent of alfalfa mixed with the clay-loam soil was almost as great as when 0.2 per cent of sodium nitrate was added alone, and 3 per cent of alfalfa mixed with the clay-loam soil produced a solvent effect 1.4 times greater than when 0.2 per cent of sodium nitrate alone was added to the soil.

#### SUMMARY

In southern California many Citrus groves are now being operated under the mulched-basin system. The principal substances employed as mulching material in the basins are stable manure, alfalfa hay, barley hay, sweet clover, and bean straw. These substances soon begin to decompose when wetted by rains or irrigation water, and the decomposition products leach into the soil. On certain soil types this method has produced very marked improvements in tree growth and fruit setting, especially when the mulches used have been alfalfa or bean straw. The present paper is concerned with an attempt to determine the extent to which the beneficial action of the decomposing organic mulches may be ascribed to a solvent action on the soil minerals, resulting in the liberation of plant food.

Soil extracts were made with organic solvents obtained from freshly decomposing alfalfa hay, sweet clover, and barley hay. The same sample of organic matter was extracted four times at intervals of from three to six weeks, the sample being kept under conditions favorable to decomposition between the intervals of extraction. These organic solvents

were used immediately for soil extraction. Two types of soil, a clay loam and a sandy loam, were used.

In the four soil extractions these organic solvents removed from the soil from two to five times as much calcium as was added to the soil with the solvents. In most cases these solvents removed more magnesium from the soil in the four extractions than was added with the solvents, the increase varying from a small fraction to about 80 per cent. The amount of iron and phosphoric acid removed from the soil by these organic solvents in the four soil extractions did not equal the total amount added to the soil with the solvents. However, the amount of iron dissolved from the soil by the organic solvents exceeded the amount dissolved by distilled water from 1 to 5.5 times. The amount of phosphoric acid dissolved from the soil exceeded the amount dissolved by water from 1.7 to 5.4 times. These various organic solvents, whether derived from a leguminous or a nonleguminous crop, had about equal solvent action on the soil minerals.

Organic solvents obtained from cow manure treated in a manner similar to the freshly decomposing green manures did not dissolve as much calcium from the soil as the solvents derived from the latter substance. They exerted about the same solvent action, however, on the other elements under investigation.

The solvent action of these organic extracts on the soil minerals appeared to be due both to the inorganic salts present in the organic solvents and to the organic compounds.

Green manures kept moist until thoroughly decomposed gave organic solvents which removed calcium from the soil in amounts several times that added with the organic solvents. These solvents also removed magnesium, phosphoric acid, and iron considerably in excess of the amount dissolved by water alone.

The organic solvents showed no alkaline reaction with phenolphthalein nor acid reaction with methyl orange.

Three per cent of green manures and stable manure mixed with soil and allowed to undergo partial decomposition increased the solubility of calcium and phosphoric acid in the soils from 30 to 100 per cent.

Artificial humus solutions free from calcium, magnesium, iron, and phosphoric acid were prepared by hydrolyzing green manures and sugar with strong acid, washing them free from acid, and extracting with ammonia. These organic solvents, when freed from ammonia, increased the solubility of calcium in the soil, compared with its solubility in water, by amounts varying from a few parts to 240 parts per million of soil. They also increased the solubility of magnesium, phosphoric acid, and iron, but to a less extent.

In brief, the solubility of calcium, magnesium, iron, and phosphoric acid in Citrus soils of the Riverside district is measurably increased by the addition of green manure, stable manure, or their extracts. This

increase in solubility is due in part to the action of the inorganic salts contained in the organic substances or their extracts and in part to the solvent action of the soluble organic compounds formed during organic decomposition. The fact that a deficiency in soluble iron is known to induce certain types of chlorosis suggests that the beneficial effects following the addition of organic matter to Citrus soils may have been in part due to its solvent action on iron and other soil compounds. Such an effect, if it exists, has not yet been differentiated from beneficial effects resulting directly from the organic material added.

# XYLARIA ROOTROT OF APPLE<sup>1</sup>

By FREDERICK A. WOLF, *Plant Pathologist*, and RICHARD O. CROMWELL, *Assistant Plant Pathologist, North Carolina Agricultural Experiment Station*

## INTRODUCTION

During the year 1913, specimens of diseased apple roots (*Malus sylvestris*) were received for examination at the North Carolina Experiment Station. These roots were found to be invaded in a characteristic manner by a fungus, which was suspected of being the cause of death of the tree from which the specimens were taken. Identification of this organism was impossible, since no fruit bodies could be found; but it was realized that the fungus exhibited points of difference from those ordinarily recognized as the cause of apple rootrots. When, however, in the spring of 1914, other specimens of the same disease were received, the fungus was isolated in pure culture by the planted-plate method. Since this fungus was very evidently different in its cultural characters from familiar forms associated with the decay of apple roots and since no report of a similar disease could be found in the literature at hand, a study of the identity and pathogenicity of this organism was begun. A preliminary report, in abstract,<sup>2</sup> of this work has appeared under the title "Black rootrot of apple." Investigations of this disease have been in progress more or less continuously for about three years, and it is deemed advisable to present at this time the data on hand relative to the distribution and symptoms of the disease and the identity, cultural characters, and pathogenicity of the causal organism.

## DISTRIBUTION OF THE DISEASE

It has been impossible thus far to make a careful survey in order to determine the exact distribution of this disease in North Carolina. Collections made by the junior author in 1914 show that the disease occurs in Haywood and Wilkes Counties. In addition, specimens were received during the same year from a correspondent in Polk County. During the following year the same disease was again collected in several localities in Wilkes County and also in the counties of Alexander, Surry, and Warren. In 1916, collections were made in Henderson County and from new localities in Wilkes and Haywood Counties. Since the places of collection are so widely separated and since no particular difficulty has been experienced in finding trees characteristically affected in any of the sections devoted to apple growing, it is believed that this rootrot is generally

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<sup>1</sup> Published with the permission of the director of the North Carolina Experiment Station.

<sup>2</sup> Fulton, H. R., and Cromwell, R. O. Black rootrot of apple. (Abstract.) *In* *Phytopathology*, v. 6, no. 1, p. 110. 1916.

present in the orchards of the western parts of the State and to some extent in other parts.

The same disease, no doubt, occurs in other States. Fulton and Cromwell<sup>1</sup> report its occurrence in Pennsylvania, and Fromme and Thomas<sup>2</sup> have reported it from Virginia.

#### LOSSES FROM ROOTROT

No approximation has been made of the losses occasioned by this rootrot. Several very obvious reasons can be mentioned to show that the assemblage of such data is next to impossible. In the first place, the symptoms in the above-ground parts of affected trees, as will be made evident later in this report, are not sufficiently characteristic to separate this rootrot from other diseases in which the root system is impaired. In order, therefore, to be certain of a field diagnosis, it is necessary to remove the soil from the collar and roots of suspected trees. Owners of orchards are generally reluctant to have any considerable number of their declining trees disturbed in this manner. In one orchard, however, 10 unhealthy trees were examined in this manner, and 5 were found to be characteristically affected. The disease was found to be generally present on a few trees in the considerable number of other orchards which were examined. It can only be said with reference to losses from this root disease that a small number of trees each year succumb in every orchard in which this trouble is present. These trees range in age from 8 to 30 years.

#### APPEARANCE OF THE DISEASE

As no part of the causal organism has been found to appear above the surface of the ground, there is no evidence of the presence of rootrot until after the disease has become well established and a considerable number of roots are involved in decay. At this stage the most prominent symptom among the above-ground organs is revealed by the foliage. If it is borne in mind that the destruction of roots proceeds gradually and that affected trees may live for several years, it will be realized that the symptoms change as the disease advances. The leaves on trees whose root systems are in intermediate stages of decay are generally quite normal in size, but are fewer in number than on healthy trees. In more advanced stages the leaves are sometimes as small as one-third the normal size. The progressive inability of diseased trees to store up sufficient reserve food very probably accounts for this production of undersized leaves. In addition, various degrees of chlorosis are always manifest.

Certain abnormalities in fruit production commonly accompany the presence of abnormal foliage. The one most generally noted is the

<sup>1</sup> Fulton, H. R., and Cromwell, R. O. Loc. cit.

<sup>2</sup> Fromme, F. D., and Thomas, H. E. The rootrot disease of the apple in Virginia. *In Science*, n. s., v. 45, no. 1152, p. 93. 1917.



occurrence of an excessively large set of small fruits, a phenomenon frequently displayed by plants weakened by disease.<sup>1</sup> In the following season such trees may produce only a few flowers, and only a score, or even a less number, of fruits are set. The apples on affected trees never develop to normal size, but ripen prematurely. Certain varieties may exhibit a reddish flush when they are no larger, perhaps, than an inch in diameter, and normal apples of the same variety on trees near by will be considerably larger and entirely green.

All of the foliage and fruit throughout affected trees usually exhibit these symptoms equally strikingly. A few cases have been observed, however, where the fruit and leaves on one or two limbs only manifested symptoms of disease; and in such cases the roots are quite normal, except on the side corresponding to the one which bears the abnormal parts.

The effect of this disease upon the twigs is indicated by a decrease in the annual increment of growth when comparison is made with healthy trees of the same variety grown under the same conditions. This decreased growth would naturally be expected to follow any impairment of the root system. In consequence of it there is a more or less marked bunching or rosetting of the leaves.

The roots of affected trees are covered with a thin, compact growth of mycelium, which is snowy white at first; after a few days, however, the superficial portion develops into a black incrustation or stroma. Under conditions favoring the optimum development of the fungus this stroma is sufficiently thick so that the outer black crust may be separated from the snowy-white web beneath without at the same time removing any of the cortical tissues of the root. Minute, black, threadlike rhizomorphs are seen to radiate from the margin of the stroma and extend for several inches along the surface of the root. These rhizomorphs may anastomose more or less, forming a network, and are in such intimate relation with the cortex that it is impossible to separate intact even small portions. These strands become obscured in the stroma, except in its most recent growth. The disintegration of the cortex beneath the stroma proceeds rapidly and follows closely upon its advance. The bark then becomes fissured, and, when it has dried, it can be readily crumbled. Affected roots are soon girdled, and the distal portions die. New roots are sometimes developed above the diseased parts, thus enabling diseased trees to live for a term of years.

Observations made in the field indicate that soil type, drainage, exposure, elevation, age, and variety of trees seem to have no bearing upon the presence of the disease, since rootrot has been found under the widest variation of these factors. Diseased trees occurred in fields that had been in cultivation for several years before the orchards were set.

<sup>1</sup> Schrenk, Hermann von. A root rot of apple trees caused by *Telephora galactina* Fr. In *Bot. Gaz.*, v. 34, no. 1, p. 65. 1902.

Trees may remain in normal health for 15 to 30 years before becoming diseased if set soon after the timber has been removed. Trees in orchards which are well cared for appear to be as subject to disease as those in neglected orchards. Not more than four trees in a group have been found to be diseased, and more often such trees stand singly. It is indicated that the dissemination of the disease is accomplished by such agencies as cultivation, rodents, and surface washing of the soil.

#### EFFECTS OF ROOTROT UPON THE WOODY TISSUES

The effects upon the woody tissues of the roots are much less profound than upon the bark and are macroscopically evident as a uniform brown discoloration. In order to secure a better knowledge of the relation of the fungus to the wood, diseased roots were embedded in celloidin, sec-

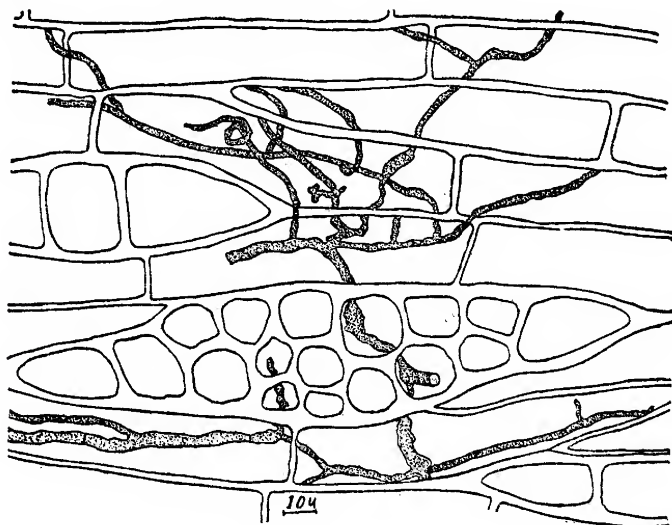


FIG. 1.—Longitudinal section of a diseased apple root. The hyphæ appear to traverse all of the xylem tissues equally well and in all directions.

tioned, and stained. The hyphæ appear to traverse all of the xylem tissues equally well and in all directions, as indicated in figure 1. They may follow the course of the vessel or medullary ray for some distance before diverging into adjacent cells. Perforation of the cell wall appears to be accomplished at any point, and no evidence has been noted that the pores serve as the places of passage. No considerable delignification occurs, as is shown by a comparison of normal and diseased wood when cross sections are treated with phloroglucin and hydrochloric acid. Starch very rapidly disappears, however, from invaded tissues, as is indicated by its absence when sections are tested with iodine. Confirmation of the digestion of starch by this fungus was secured by growth on starch agar. A halo extending beyond the margin of the colony resulted on this medium, thus affording an ocular demonstration of the excretion of amylase. Further evidence of the activity of this organism was

sought by the cultural methods employed by Crabill and Reed <sup>1</sup> in which the carbon-containing compounds cellulose, amygdalin, fibrin, albumin, peptone, casein, and asparagin were added to stock agar of inorganic salts. Although the fungus grows slowly on nutrient agar, well-defined halos had formed within a few days on casein and fibrin agar. Asparagin agar, acidified with hydrochloric acid and made yellow by the addition of rosolic acid, is changed to red by ammonia liberated in the decomposition induced by the organism. The growth on cellulose, amygdalin, and albumin agar indicates that these compounds are not utilized by the fungus. Even though a good growth on peptone resulted, no halo was formed. The results with casein, fibrin, and asparagin indicate that certain proteolytic enzymes (erepsin, protease, and amidase) are secreted.

#### CAUSE OF THE DISEASE

Isolations have been made at various times from diseased roots from several sources which have constantly yielded an organism with a very characteristic mycelial growth. These cultures remained sterile until the summer of 1915, when conidial fructifications appeared in certain of them. It was not definitely proved, however, that these conidia were those of the causal organism until several months later. Stromatic arms, like those of certain species of *Xylaria*, formed in these cultures (Pl. 3). The conidia, too, not unlike those of species of this genus, were formed either on these arms or on elevations arising from the incrustation on the surface of the culture medium.<sup>2</sup>

The conidia are hyalin, elongated oval in outline, with a blunt truncate pedicel, and measure about 10 by 3 to 3.5  $\mu$ . They are borne singly as lateral buds from the sporogenous hypha (fig. 2, *b*). As yet all attempts to germinate them have been fruitless.

Perithecia have never appeared in any of these cultures, even though a variety of media have been employed under several sets of environmental conditions. Perithecial stromata have been found, however, upon the roots of trees which had succumbed to rootrot. Thus far, all attempts to germinate the ascospores have been unsuccessful, so that it has been impossible to determine by growth in culture or by inoculations

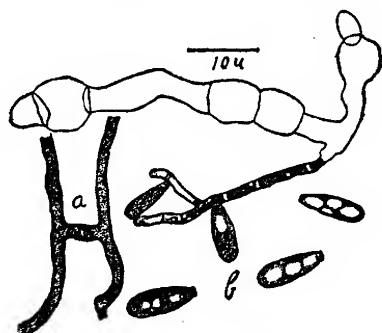


FIG. 2.—*a*, *Xylaria* sp.: Hyphae, showing the fusion occasionally noted. *b*, The conidia are hyalin, elongated oval in outline, with a blunt truncate pedicel, and measure about 10 by 3 to 3.5  $\mu$ . They are borne singly as lateral buds from the sporogenous hypha.

<sup>1</sup> Crabill, C. H., and Reed, H. S. Convenient methods for demonstrating the biochemical activity of microorganisms, with special reference to the production and activity of enzymes. In *Biochem. Bul.*, v. 4, no. 13, p. 30-44, pl. 1, 1915.

<sup>2</sup> Specimens from cultures were submitted for examination to Prof. George F. Atkinson, who stated that the organism was probably a species of *Xylaria*.

whether this perithecial form is genetically connected with the conidial stage which appeared in culture. The perithecial material is morphologically quite like *Xylaria hypoxylon*, a species in which is included a wide variety of forms. It is quite probable that several species of *Xylaria* are involved in this disease, as has been suggested by Fromme and Thomas,<sup>1</sup> who found *Xylaria polymorpha* associated with apple rootrot in Virginia.

#### CULTURAL CHARACTERS

Various kinds of nutrient agar were found to be unfavorable substrata, since on them growth proceeded slowly and there was only a slight tendency toward the formation of a black incrustation and rhizomorphs.

When agar added to decoctions of apple fruits or roots was employed, and also when sterilized, well-moistened apple roots were used as a culture medium, an abundant mycelium and a well-developed incrustation were produced. No fructifications have been noted on these media, however, even in cultures which have been kept growing for three years.

The general appearance of the mycelium on various culture media is quite like that on decaying apple roots. The young filaments (fig. 3, *b, d*) are hyalin, branched, granular, and highly refractive, with an average diameter of only 2 to 3  $\mu$ . Considerable variation in the diameter of the filaments occurs, as indicated in figures 2, *b*, and 3, *a*.

Later, the hyphæ lose their granular contents, become brown to olivaceous in color, and numerous blunt, spinulose terminals are developed (fig. 3, *c, f*). Filaments in old cultures become more closely septate, the cells may attain a diameter of 4 to 8  $\mu$  (fig. 2, *b*) and are chlamyospore-like, in that they retain their vitality even after desiccation for several months. Fusion of hyphæ, as shown in figures 2, *a*, and 3, *e*, has occasionally been noted.

On potato plugs, steamed corn meal in flasks, bean pods, and other sterilized plant parts, a copious mycelial growth is produced, which may become crustlike; and, in addition, stromatic arms and conidia are formed. Conidial formation, in reddish brown or grayish sporodochia-

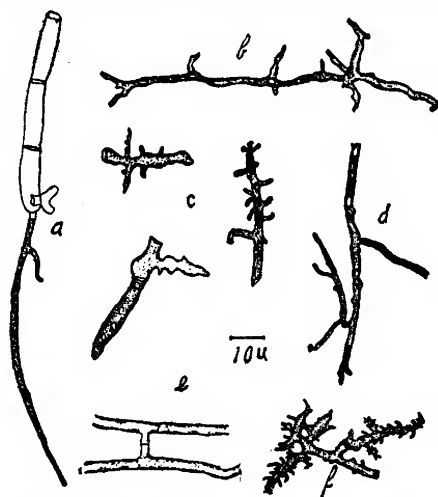


FIG. 3.—*Xylaria* sp.: Mycelium, showing (a) considerable variation in the filaments; *b, d*, the young filaments are hyalin, branched, granular, and highly refractive, with an average diameter of 2 to 3  $\mu$ ; *c, f*, the hyphæ later lose their granular contents, become brown to olivaceous in color, and numerous blunt spinulose terminals are developed; *e*, fusion of hyphæ.

<sup>1</sup> Fromme, F. D., and Thomas, H. E. Loc. cit.



like masses, occurs most profusely on the incrustation on corn meal. Bean pods or corn meal in flasks appear to be most favorable for the formation of stromatic arms. These stromatic arms vary in size from very small to those having a length of 10 to 12 cm. and a diameter of 1 cm. They are either entire or branched in a coralloid manner. The stromata are flesh-colored within and are generally covered, except at the tip, which is also flesh-colored, with a dense hirsute coating. These hairs are 3 to 5 mm. long and impart to the stromata a beautiful variety of colors, among which are gray and shades of brown, violet, and green. Small stromata may not possess this coating of hairs.

A study has been made of the influence of temperature, light and darkness, and moisture upon the growth of this organism. For this purpose, several sets of cultures were prepared, one of which was maintained in an incubator at 37° C. Scarcely any growth takes place at this temperature. A moderate growth of mycelium, conidia, and stromatic arms 4 to 6 cm. long were produced in another set kept at 28°. Room temperatures of 21° to 25° were found to be more favorable for growth than this last temperature, but the most luxuriant growth occurred in cultures kept in an ice box at temperatures of 11° to 13°. The largest stromatic arms obtained were formed at this temperature.

Light appears to exert no morphogenic stimulus in the formation of stromatic arms, since these structures, both simple and branched, appeared in sets of cultures kept in a photographic dark room. The vegetative growth, too, appears to be as luxuriant in darkness as in the light.

In the tests upon the influence of moisture, flask cultures containing 12 gm. each of corn meal were employed. This corn meal was moistened by the addition of quantities of water varying from 20 to 65 c. c. After inoculation the cultures were incubated for six weeks at room temperature. As judged by the luxuriance of growth, 40 to 50 c. c. of water appear to be the optimum quantity. No conidia formed in the cultures containing less than 40 c. c. of water.

#### PATHOGENICITY

Four apple trees only were used in making tests to determine the parasitism of this organism. On one tree three inoculations were made; on each of two others, six; and on the other, ten. These inoculations were made at West Raleigh, N. C., during the months of May, June, and September. The soil was first removed so as to expose the roots, after which the inoculations were effected. The inoculum, which consisted of mycelia from steamed-rice cultures, was either inserted into wounds made by scraping off the cortex, or by making incisions into it, or was applied to uninjured roots. The places of inoculation were then covered with a layer of soft paraffin. The roots inoculated varied in diameter from  $\frac{3}{16}$  to 3 inches. Infections were uniformly successful, irrespective



of whether or not the tissues were injured. The progress of the disease, however, varied considerably. In some cases small areas only had become involved in decay within six weeks, and in others the disease had advanced several inches to a foot or more beyond the point of inoculation. Even though relatively few inoculations have been attempted, the evidence in hand shows beyond doubt that the organism is to be regarded as a vigorous pathogene.

#### SUMMARY

(1) A little-known apple rootrot which causes the death of trees has been more or less continuously investigated for the past three years.

(2) It has been observed to occur in six widely separated counties in North Carolina and in all probability is the same disease which has been observed in sections of Virginia and Pennsylvania.

(3) The symptoms manifested by the above-ground parts of affected trees do not serve to distinguish this disease from other apple rootrots.

(4) The roots, however, are characteristically covered with black fungus incrustations from whose margins radiate minute, black rhizomorphs. The cortex is quickly corroded, and the roots are girdled while disintegration of the woody portions proceeds slowly.

(5) Isolations have constantly yielded a form whose conidial fructifications and stromatic arms indicate its relationship to *Xylaria* spp.

(6) The ascigerous stage of a species of *Xylaria* has been found upon diseased apple roots, but has not been proved to be connected with the conidial stage developed in artificial culture.

(7) Mature mycelium in culture possesses numerous characteristic spinulose branches.

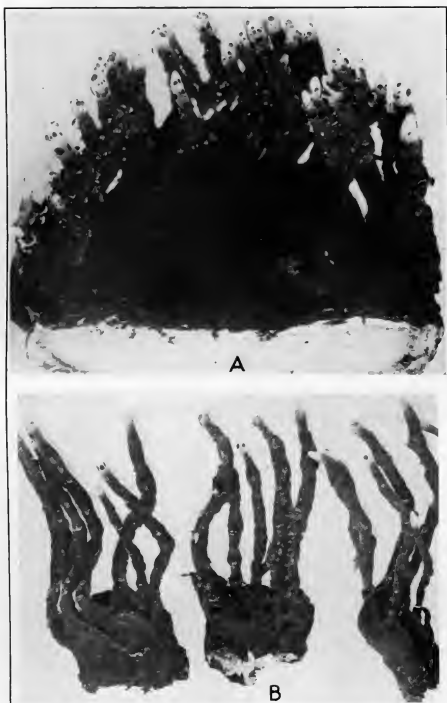
(8) The optimum development of the organism seems to be obtained when temperatures of 11° to 13° C. are maintained either in the presence or absence of light.

(9) Pathogenicity is established by inoculation with pure cultures into the roots of living apple trees.

PLATE 3

A.—Stromatic arms formed in cultures on steamed corn meal; resembling those of certain species of *Xylaria*. They may form in abundance on this medium. Excreted drops give the arms a scarred appearance. About natural size.

B.—A few stromatic arms from the same culture. About natural size.



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## COMPARISON OF THE HOURLY EVAPORATION RATE OF ATMOMETERS AND FREE WATER SURFACES WITH THE TRANSPIRATION RATE OF MEDICAGO SATIVA

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### INTRODUCTION

The rate of evaporation from a free water surface or from a moist porous surface is usually considered the best single-valued expression of the intensity of the weather factors influencing transpiration. Such a relationship is, however, subject to the uncertainty arising from the marked differences in the energy-absorbing and energy-dissipating properties of the transpiring and evaporating surfaces. It is evident that the transpiring and evaporating surfaces must be in agreement in this respect if the departure of transpiration from evaporation during the day is to be taken as evidence of a change in the transpiration coefficient, resulting from stomatal control or other reversible changes within the plant body.

Fluctuations in transpiration from day to day appear to be reflected with approximately the same degree of fidelity by a number of widely different forms of evaporating surfaces, provided precautions are taken to maintain the uniformity of these surfaces throughout the period of observation. When the hourly transpiration rate is under consideration, however, the individuality of the evaporating surface to which the transpiration is referred can not be ignored. It is this phase of the question that forms the subject of the present paper.

### APPARATUS AND METHODS

**ATMOMETERS.**—Four types of porous-cup atmometers as designed by Livingston (1915), including white cylinders, brown cylinders, white spheres, and white Bellani plates, were employed in the measurements (Pl. 4).

Each type was represented by four atmometers<sup>1</sup> which were weighed independently at two-hour intervals throughout the day. The atmometers were mounted on 500-c. c. bottles closed with rubber stoppers containing a small air vent, and were freely exposed in racks about 1 meter above the ground. Distilled water was employed in all the measurements. All the atmometers used were new, and each cup was inverted and flushed with water for several hours before it was set up. The cups were set up for 36 hours before the detailed measurements were begun.

**EVAPORATION TANKS.**—Two evaporation tanks of widely different types were also employed in the measurements, one containing a layer of water approximately 1 cm. in depth, while the depth of the water in the other was about 50 cm.

The shallow tank, which was 91 cm. in diameter and 2.5 cm. high, was supported on a wooden disk 4 cm. in thickness and mounted about 1 meter above the ground on an automatic scale. The tank was blackened inside. The depth of water was automatically maintained at about 1 cm. by means of a Mariotte apparatus mounted on the scale platform<sup>2</sup> (Pl. 5). The automatic scale was sufficiently sensitive to record the loss of a layer of water 10  $\mu$  (0.01 mm.) in thickness.

The deep evaporation tank, which was 192 cm. in diameter and 60 cm. in depth, was sunk in the ground to within 10 cm. of the top. This tank was equipped with a continuously recording gauge of the float type reading to 0.1 mm.

**FILTER-PAPER EVAPORIMETER.**—An evaporimeter of special design, in which the evaporating surface was a saturated filter paper (12.5 cm. in diameter) was also employed in the measurements (Pl. 6). The filter paper was supported on a light flat brass disk, with a rim 1 mm. in height. The paper was kept saturated by means of a Mariotte apparatus connected with a tubulure on the lower side of the disk. This arrangement avoids the drying out of the filter paper, which is sometimes encountered in the Piche evaporimeter, due to the failure of the instrument to feed properly. The evaporation was determined by weighing the whole apparatus at two-hour intervals.

<sup>1</sup> The identification numbers and the coefficients of the atmometers used in the comparisons were as follows:

	No.	Coefficient.		No.	Coefficient.
White cylinders.....	5-36	0.77	White spheres.....	16-16	0.94
Do.....	5-37	.77	Do.....	16-77	.94
Do.....	5-57	.77	Do.....	16-105	.94
Do.....	5-142	.77	Do.....	16-146	.94
Brown cylinders.....	4-17	.74	White Bellani plates.....	D-137	.....
Do.....	4-25	.74	Do.....	D-175	.....
Do.....	4-41	.76	Do.....	D-205	.....
Do.....	4-59	.74	Do.....	D-206	.....

<sup>2</sup> This equipment is the same as that used by the writers in connection with earlier transpiration measurements (Briggs and Shantz, 1915, 1916).



TRANSPIRATION MEASUREMENTS.—The transpiration measurements were made on automatic scales of the type previously described (Briggs and Shantz, 1915). Grimm alfalfa (*Medicago sativa* L.) was employed in the measurements. The plants were growing in the large sealed pots used in the writers' water-requirement measurements, and had been carried over from the preceding year. The plants were fully exposed to the sun and were coming into bloom during the measurements, the second cutting of the season being made at the close of the experiments. The green weights of the crops and the total plant surface<sup>1</sup> were as follows:

Pot No.	Green weight.	Total plant surface.
	Gm.	Sq. cm.
301	258	15,700
303	287	17,400

SUBSIDIARY MEASUREMENTS.—In addition to the above measurements, the solar-radiation intensity, air temperature, wet-bulb depression, and wind velocity were recorded simultaneously by means of automatic instruments which have already been described (Briggs and Shantz, 1916).

REDUCTION OF DATA.—The data for the white cylinder, brown cylinder, and white-sphere atmometers have been corrected by means of the coefficients supplied with the instruments. No corrective factor has been applied to the Bellani plates. The data for the filter-paper evaporimeter have been reduced to an area of 100 sq. cm. and the data for shallow evaporation tank to an area of 1 square meter.

#### EXPERIMENTAL RESULTS

The measurements were made at Akron, Colo., in July, 1916, during a period of hot, dry weather. The data representing the hourly evaporation rate from the various surfaces, the transpiration rate, and the weather conditions are given in Table I. Each atmometer determination is the mean of four independent measurements. The transpiration is represented by the mean of two pots measured independently. The data are presented graphically in figure 1.

The hourly transpiration is plotted at the top of the figure, followed by the evaporation from the different types of atmometers and from the free water surfaces. It will be noted that the different atmometers, although varying widely in form (Pl. 4), give graphs which are similar in their characteristics. This is brought out more clearly in figure 2, in which the ratio of the transpiration rate to the evaporation rate for each of the various types of atmometers is plotted hour by hour.

<sup>1</sup>The determinations of plant surface are based upon the measurement of the green weight and total surface of a representative plant.

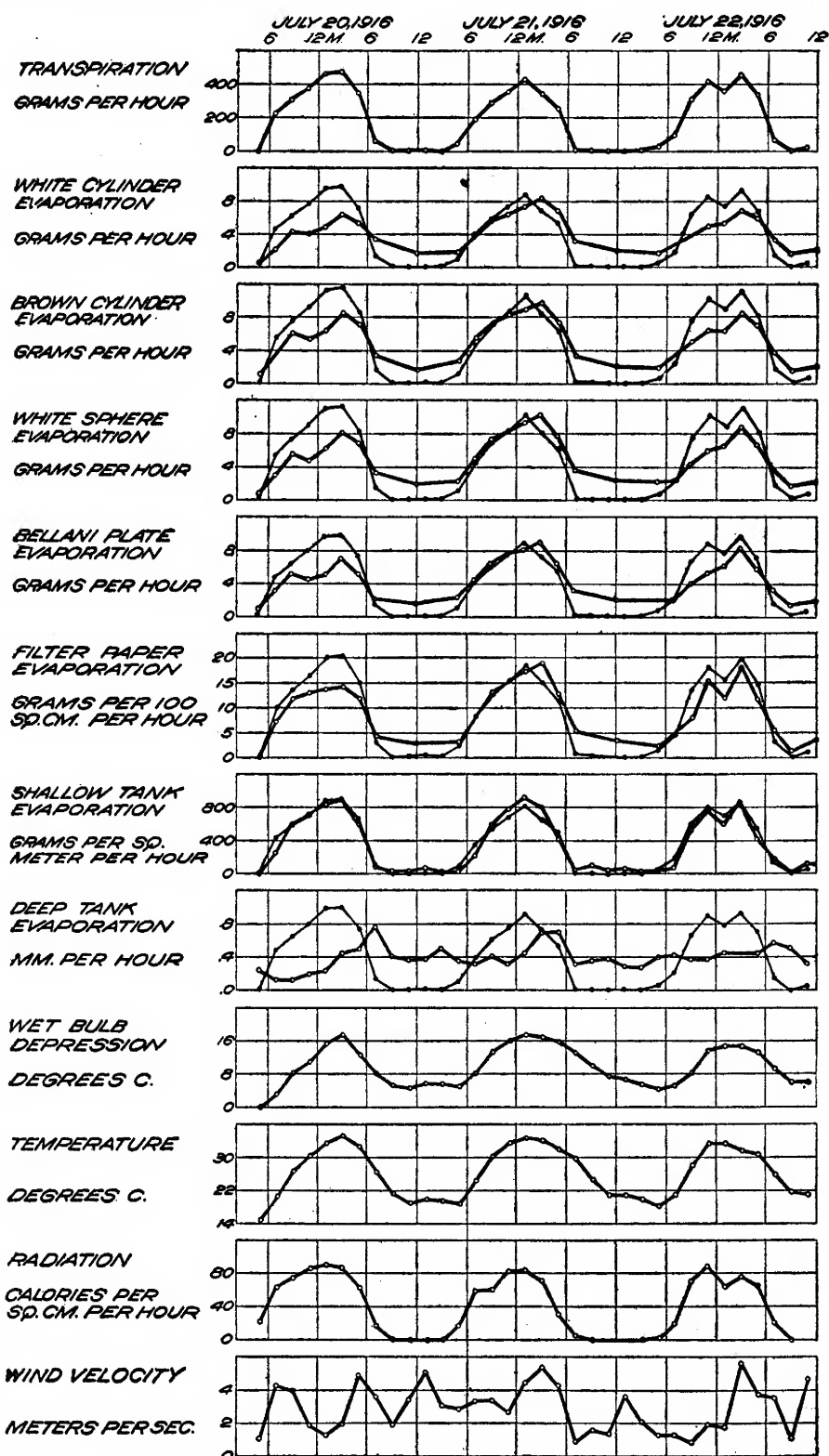


FIG. 1.—Graphs showing the hourly transpiration rate of alfalfa, the hourly evaporation rate from different surfaces, and the weather conditions during a three-day period at Akron, Colo. The heavy lines (with points marked by circles) represent the observed data. The light lines (with points marked by dots) represent the transpiration rate, with the scale of ordinates so chosen that the area under the transpiration graph is equal to the area under the accompanying evaporation graph.

TABLE I.—Hourly evaporation rate from atmometers and free water surfaces, the hourly transpiration of *Medicago sativa*, and the hourly intensity of weather factors

Item.	A. M.						P. M.						Average, 8 p. m. to 4 a. m.
	12 to 2	2 to 4	4 to 6	6 to 8	8 to 10	10 to 12	12 to 2	2 to 4	4 to 6	6 to 8	8 to 10	10 to 12	
July 20, 1916.													
Transpiration of alfalfa..... gm. per hour.						380	405	475	350	65	5	5	6
Evaporation:													
White cylinder..... do.			.5	2.2	4.3	4.0	4.9	6.4	5.4	3.4			1.7
Brown cylinder..... do.			1.1	3.7	6.1	5.4	6.4	8.5	7.1	3.4			1.7
White sphere..... do.			.7	3.1	5.5	4.8	6.2	8.1	7.0	3.2			1.9
Bellani plate..... do.			.8	3.1	5.2	4.5	5.1	7.0	5.2	2.1			1.5
Filter paper, gm. per 100 sq. cm. per hour.			.0	7.0	11.5	12.7	13.5	14.2	11.8	4.1			2.7
Shallow tank, gm. per sq. meter per hour.				260	600	725	845	900	600	95	40	40	50
Deep tank..... mm. per hour.			.25	.13	.13	.19	.24	.45	.51	.76			
Wet-bulb depression..... °C.			.0	3.3	8.3	11.1	15.6	17.5	12.8	8.3	5.3	4.7	
Radiation, calories per sq. cm. per hour.	0	0	22	63	75	85	90	87	63	18	0	0	
Temperature..... °C.	20.6	20.6	15.0	20.6	20.7	30.5	33.6	35.3	32.8	26.7	21.4	19.2	
Wind velocity..... meters per second.			1.1	4.3	4.0	1.9	1.3	2.0	4.9	3.6	1.9	3.4	
July 21, 1916.													
Transpiration of alfalfa..... gm. per hour.	10	5	50	190	290	355	430	345	260	10	5	0	3
Evaporation:													
White cylinder..... do.			1.9	4.0	5.6	6.4	7.3	8.3	6.9	3.1			2.0
Brown cylinder..... do.			2.8	5.5	7.2	8.3	8.9	9.7	7.3	3.3			2.1
White sphere..... do.			2.3	5.0	7.3	8.3	9.3	10.3	7.8	3.6			2.4
Bellani plate..... do.			2.3	4.4	6.3	7.5	8.0	8.9	6.4	3.1			2.0
Filter paper, gm. per 100 sq. cm. per hour.			3.0	8.2	12.9	15.1	17.2	18.8	12.7	5.3			3.2
Shallow tank, gm. per sq. meter per hour.	75	40	40	230	590	780	920	800	435	55	105	50	65
Deep tank..... mm. per hour.	.38	.51	.36	.32	.41	.33	.45	.70	.70	.32	.36	.38	
Wet-bulb depression..... °C.	5.8	5.8	5.0	8.3	13.6	16.1	17.5	16.9	15.8	13.3	10.0	7.5	
Radiation, calories per sq. cm. per hour.	0	0	18	59	61	83	84	72	31	5	0	0	
Temperature..... °C.	20.0	19.7	18.9	24.5	30.3	33.6	34.7	34.2	32.0	29.7	24.5	21.1	
Wind velocity..... meters per second.	5.1	3.1	2.9	3.4	3.4	2.7	4.5	5.4	4.3	.9	1.6	1.4	
July 22, 1916.													
Transpiration of alfalfa..... gm. per hour.	5	0	30	100	345	420	365	455	335	70	0	25	5
Evaporation:													
White cylinder..... do.			1.7	1.8	3.3	4.9	5.3	6.7	5.8	3.3	1.6		2.1
Brown cylinder..... do.			1.9	2.3	5.1	6.3	6.3	8.5	7.0	3.6	1.5		2.0
White sphere..... do.			2.2	2.3	4.3	5.9	6.5	8.8	6.6	3.7	1.7		2.2
Bellani plate..... do.			1.9	1.9	3.9	5.2	5.9	8.1	5.6	3.1	1.2		1.7
Filter paper, gm. per 100 sq. cm. per hour.			2.1	4.9	7.8	15.2	11.9	18.0	11.5	5.3	1.2		3.6
Shallow tank, gm. per sq. meter per hour.	75	30	30	75	535	760	600	865	430	185	15	115	60
Deep tank..... mm. per hour.	.29	.28	.39	.42	.38	.38	.45	.45	.45	.57	.51	.32	
Wet-bulb depression..... °C.	6.7	5.6	4.4	5.3	8.3	13.6	14.7	14.7	13.3	9.4	6.1	6.1	
Radiation, calories per sq. cm. per hour.	0	0	2	20	71	89	65	77	66	21	0	0	
Temperature..... °C.	21.1	20.0	18.3	21.1	21.1	33.3	33.3	31.7	30.8	25.8	21.7	21.1	
Wind velocity..... meters per second.	3.6	2.1	1.3	1.3	.9	2.0	1.8	5.6	3.8	3.6	1.1	4.7	

The transpiration graph has also been superimposed on each of the evaporation graphs in figure 1, choosing the scale of ordinates in each instance so that the total area under the transpiration graph for the three days is equal to the area under the evaporation graph. This affords a graphical comparison of the fidelity with which the transpiration is reflected in the hourly evaporation rate from the different instruments.

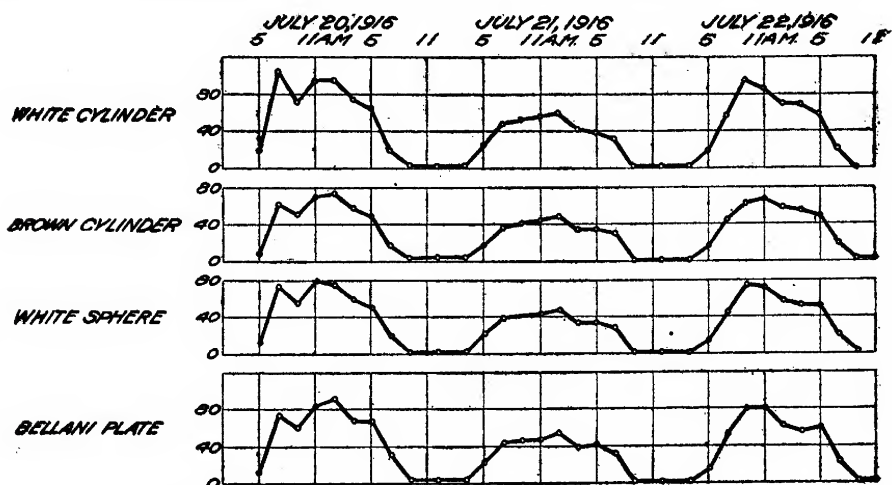


FIG. 2.—Ratio of the transpiration rate to the evaporation rate for each of the various types of porous-cup atmometers, plotted hour by hour.

#### MEAN HOURLY DEPARTURE OF EVAPORATION AND TRANSPIRATION

A quantitative expression of the relative value of the several instruments in predicting the hourly transpiration may be obtained by determining the average departure of the evaporation graph from the transpiration graph, the mean departures being expressed in percentage of the mean hourly evaporation in each instance. The calculation has been based both on the total period covered in the measurements and on the three daylight periods from 6 a. m. to 6 p. m. These computations are presented in Table II. The error involved in predicting the transpiration rate from the evaporation measurements is indicated by the figures in the last column of the table.

TABLE II.—Average hourly departure of evaporation rate from transpiration rate in percentage of the mean evaporation rate for various types of atmometers and for free water surfaces

Type of atmometer.	Day periods, 6 a. m. to 6 p. m.	Total period.
	<i>Per cent.</i>	<i>Per cent.</i>
White cylinder.....	38	49
Brown cylinder.....	29	41
White sphere.....	31	43
Bellani plate.....	30	41
Filter-paper evaporimeter.....	22	31
Shallow tank.....	12	17
Deep tank.....	93	89

The deep tank gives little indication of the hourly transpiration rate, since the average departure in this case amounts to about 90 per cent of the mean evaporation rate. In other words, the evaporation from the deep tank at different hours of the day is not proportional to the transpiration of the plant.

The average hourly departure in the case of the white cylinders amounts to about 50 per cent of the mean hourly transpiration. In other words, the average error in predicting the amount of transpiration at each hour in the day from the evaporation when the ratio of daily transpiration of the plant to the daily evaporation of the atmometer is known would amount to one-half the mean hourly value of the transpiration. When the brown cylinder, Bellani plate, or white sphere is used, this error is reduced to about 40 per cent. In the case of filter-paper evaporimeter the error is reduced to about 30 per cent. The best results were obtained with the shallow, blackened tank, the error being less than 20 per cent.

Since the night values of transpiration and evaporation are low and the greater part of the evaporation and transpiration occurs during the daylight hours, the writers have also computed the mean departure of each of the different instruments on the basis of the daylight hours only. The departures, with the exception of the deep tank, are, in this case, about two-thirds of those for the total period. The evaporation rate of the shallow, blackened tank during the daylight hours again follows the transpiration graph more closely than that of any of the other instruments, the mean departure being only about two-fifths that observed in the case of the atmometers. The filter-paper evaporimeter also shows a lower mean departure than the atmometers. Of the latter instruments, the brown cylinder, white sphere, and Bellani plate are practically identical and show a departure of about 30 per cent, while the white cylinder shows a somewhat greater mean departure. The similarity of the results from the three first-named types is remarkable when the difference in form and color are considered.

The evaporation from the deep tank shows practically no correlation with the transpiration when the hourly values are considered. Attention has already been called to this discrepancy, which results from the storage of heat energy in the great mass of water during the day and its slow dissipation through evaporation during the night. The maximum in the evaporation graph from the deep tank occurs in the late afternoon or early evening.

The fact that an evaporating surface shows a low correlation with the hourly transpiration does not necessarily imply a correspondingly low correlation on a daily basis. This is demonstrated in the case of the deep tank, which showed in 1914 (a dry year) a correlation with transpiration of  $0.63 \pm 0.01$  (Briggs and Shantz, 1916a), while the correlation of the shallow tank for the same period was  $0.72 \pm 0.01$ . The evaporation from the deep tank probably represents approximately the distribution of the



loss of water from a large body of water, heat energy being stored in both systems during the day. The transpiration graph of alfalfa may be assumed to represent approximately the distribution of the hourly loss of water from any actively growing vegetative cover. The hourly loss from large bodies of water thus appears to be more nearly uniform throughout the 24-hour period, while the loss from land areas covered with vegetation is confined almost wholly to the daylight hours and largely to the midday period.

#### INFLUENCE OF WIND VELOCITY AND SOLAR-RADIATION INTENSITY ON EVAPORATION

It is of interest to consider the departure of the various evaporation graphs from the transpiration graph in relation to the intensity of the several climatic factors. It will be noted that all the atmometer graphs showed a relatively low evaporation from 10 a. m. to 4 p. m. on July 20 compared with either the transpiration or the shallow-tank evaporation. The explanation of this may be found in the wind velocity, which is relatively low during this period, being less than 2 meters per second (4 miles per hour). The relatively high evaporation from the atmometers from 2 to 6 p. m. on the following day (July 21) is also explainable on the basis of the high wind velocity during this period. The maximum evaporation from the atmometers on the third day also coincides with the period of maximum wind velocity. It is evident, therefore, that the evaporation from the atmometers is affected to a much greater degree by variations in wind velocity than either the transpiration or the evaporation from the shallow tank.

On the other hand, the atmometers are less sensitive to changes in solar radiation than either the plant or the shallow tank, as is shown by the afternoon readings on July 22. Both the transpiration graph and the shallow-tank evaporation graph show maxima at 11 a. m. and 3 p. m. on this date, due to a temporarily clouded sky from 12 noon to 2 p. m. The radiation on July 22 was also deficient from 7.45 a. m. to 11 a. m. due to the passing of cumulus clouds. This reduced the transpiration and shallow-tank evaporation and brought the transpiration graph more nearly into conformity with the atmometer graphs during this period.

#### ATMOMETER CALIBRATION AND OBSERVATION ERRORS

Since four instruments of each type were used in the atmometer readings, it is possible from the results to determine the calibration error and the probable error of a single observation for each individual atmometer. To each determination was applied the calibration correction which accompanied the instrument (see footnote, p. 278). The ratio of the individual observation of each instrument to the mean

of the four instruments was then taken. Since each of the instruments had been calibrated, the departure of the mean of this series of ratios from unity represents the error in the calibration coefficient. These departures, which are given in the third column of Table III, show errors ranging from +4 to -2 per cent for the white spheres, from +3 to -4 per cent for white cylinders, and from +2 to -2 per cent for the brown cylinders.

TABLE III.—*Calibration error and probable error of single observation for individual atmometers*

Atmometer.	Ratio to mean.	Observed error in coefficient.	Probable error of single observation.
<b>White cylinder:</b>		<i>Per cent.</i>	<i>Per cent.</i>
5-36.....	1.02	+2	±3.7
5-37.....	.96	-4	±1.7
5-57.....	1.03	+3	±1.9
5-142.....	.98	-2	±2.7
<b>White sphere:</b>			
16-16.....	.98	-2	±2.3
16-77.....	.98	-2	±1.7
16-105.....	1.00	0	±1.6
16-146.....	1.04	+4	±3.2
<b>Brown cylinder:</b>			
4-17.....	1.01	+1	±2.6
4-25.....	.98	-2	±1.5
4-41.....	1.02	+2	±2.5
4-59.....	.98	-2	±2.6
<b>Bellani plates:</b>			
D-137.....	.94	.....	±3.0
D-175.....	.96	.....	±1.8
D-205.....	1.03	.....	±2.6
D-206.....	1.07	.....	±3.2

The probable error of a single observation of each of the instruments has also been computed for each instrument. The results are given in the last column of the table. These errors, which do not include the errors in the coefficient, amount to from 2 to 4 per cent. It is possible from these data to obtain an expression of the degree of reliance to be placed upon individual observations. Determinations with a single atmometer at 2-hour intervals during the day thus appear to be subject to a probable error of about 5 per cent,<sup>1</sup> if the weighings are accurate to 0.1 gm. This probable error refers only to calibration uncertainties and to so-called "accidental" errors, as exemplified by the variation of the individuals in a group of atmometers of the same type. It does not include, for example, errors arising from the possible failure of the whole group of atmometers to respond freely to changes in their environment.

<sup>1</sup> Based upon the square root of the sum of the squares of the mean values of the calibration error and of the observation errors, taken without regard to sign.

## RATIO OF TRANSPIRATION TO EVAPORATION

The ratio of the transpiration rate to the evaporation rate from an atmometer should give a straight line when plotted hour by hour if the two systems respond alike to changes in environment. The experimental graph is, however, periodic in form, exhibiting a marked minimum during the night hours.

Fluctuations in the hourly transpiration-evaporation ratio as represented in such a graph have been interpreted (Livingston, 1906, 1913; Livingston and Hawkins, 1915; Edith Shreve, 1914 and 1915; Forrest Shreve, 1914; Burns, 1915) as representing departures in the transpiration rate of the plant from that which would prevail if the plant responded freely to its environment. This point of view implies the assumption that an atmometer responds perfectly to its environment; in other words, that the evaporation rate from the atmometer affords a perfect summation of the environmental conditions determining evaporation from a plant surface. A departure in transpiration from that indicated by this summation would then, according to this viewpoint, be taken as evidence of a change in the transpiration coefficient resulting from stomatal control or from other reversible changes within the plant body.

The correctness of the assumption relative to the perfect response of the porous atmometers must be questioned when the hourly evaporation rate from the atmometer is compared with that from the shallow tank. During the night, for example, the evaporation from the atmometers is relatively much higher than from the free water surface. If the atmometer is accepted as responding freely to its environment, then the free water surface, even under conditions of low evaporation, can not be considered as a free evaporating system.

The same question as to the free response of the porous atmometers to their environment arises when the relative transpiring power, as expressed by the transpiration-evaporation ratio, is compared with the "index of transpiring power" as measured by the cobalt-paper method. Livingston (1913, p. 24) has in fact found in comparing the hourly graphs obtained by the two methods that the—

relative transpiration ratio as determined either by the brown or white atmometer presents a much flatter graph than that indicated by the hygrometer index of transpiring power.

It is evident, therefore, that one of these instruments must fail to respond freely to the intensity of the environment.

The ratio of the transpiration rate of *Medicago sativa* to the evaporation rate of each of the different types of atmometers is presented graphically in figure 2. The graphs are markedly similar in form when the wide differences in the porous atmometers are considered. The changes from hour to hour in any one graph are, with few exceptions, reflected in all the others, and such differences as exist are, in most cases, comparable

with the probable error. In other words, the relative evaporation rate through a 24-hour period is practically the same whether the porous evaporating surface is in the form of a closed vertical cylinder, a sphere, or a flat horizontal plate. The brown cylinder, owing to its higher evaporation rate during the daylight hours, reduces the ordinates of the ratio graph; but otherwise this graph is also similar in form to those obtained with the white porous surfaces. When the transpiration of the plant is referred to the evaporation from a free water surface in a shallow blackened tank, a different form of transpiration-evaporation graph is obtained, which shows less daily fluctuation than when the transpiration is referred to the porous atmometers.

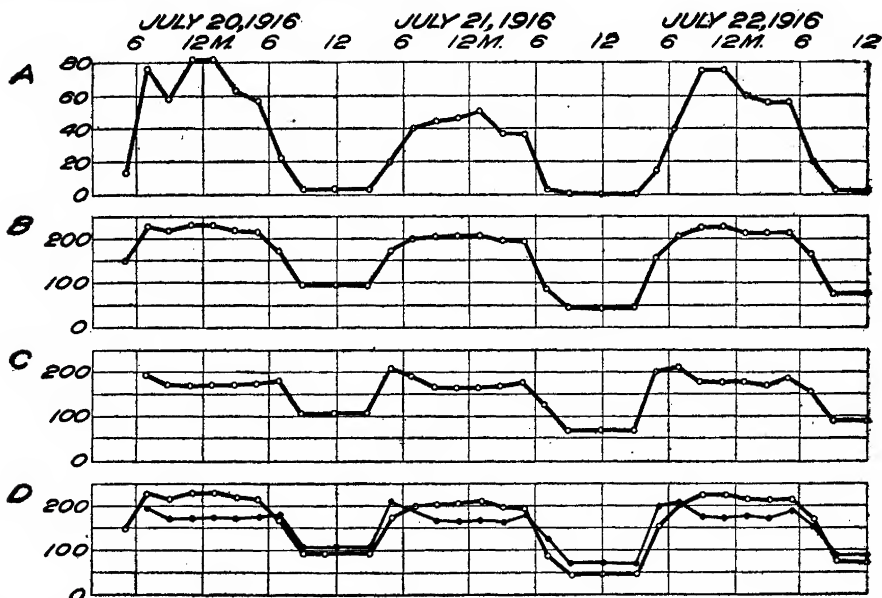


FIG. 3.—Graphs showing the transpiration-evaporation ratio: A, The ratio of the hourly transpiration to the hourly mean evaporation from the atmometers. B, Graph A with ordinates plotted logarithmically. C, The ratio of the hourly transpiration to the hourly evaporation from the shallow tank, with ordinates plotted logarithmically. D, Graphs B (heavy line) and C (light line) superimposed.

These two types of graphs are compared in figure 3. The first graph (A) of the figure represents the ratio of the transpiration rate to the mean evaporation rate of the four types of atmometers (16 instruments in all). This is the form in which the transpiration-evaporation graph is usually presented. It is however preferable, in the opinion of the writers, to plot the logarithms of the ratios, as this avoids the distortion of the graph, especially when ratios on both sides of unity are being considered. In the second graph (B) in figure 3, the transpiration-evaporation ratio, as given in the first graph, is plotted logarithmically. The third graph (C) represents the transpiration-evaporation ratio when the evaporation from the shallow tank is used as a basis of reference, and is also plotted logarithmically. At the bottom of figure 3 the last two graphs (B

and C) are superimposed. It will be noted that the fluctuation in the ratio throughout the 24-hour period, as well as the mean hourly departure, is decidedly less when the transpiration is referred to the evaporation from the shallow, blackened tank. In other words, the transpiration of *Medicago sativa* follows the evaporation from the shallow, blackened tank more closely than that from the porous atmometers.

It is evident, however, that an evaporating system has not yet been secured that responds to its environment in the same way as the plant. Both the atmometer and the tank depart widely from the plant during the night under the conditions prevailing in the Great Plains. Reference to the logarithmic graphs will show that the transpiration-evaporation ratio when referred to the evaporation from the shallow, blackened tank is practically constant from 8 a. m. to 4 p. m., the period during which the plants are transpiring most rapidly. In *Medicago sativa*, at least, there is no evidence of a change in the transpiration coefficient during this period. The higher ratio obtained in the early morning and late afternoon hours is perhaps explainable by the difference in exposure of the plants and the free water surface. The isolated plants are exposed to the normal rays of the sun practically throughout the day while the tank receives only the vertical component of radiation. Where vegetation is massed under field conditions, the plants likewise are exposed for the most part only to the vertical component of radiation.

The transpiration measurements in these experiments are confined to *Medicago sativa*, and it is possible that the transpiration rate of other plants under the same conditions would have given transpiration graphs differing in form from those obtained. Earlier measurements (Briggs and Shantz, 1916, p. 638) have shown, however, that in the case of rye and amaranthus, the hourly transpiration rate is correlated with the evaporation rate from the shallow tank to a degree comparable with the corresponding correlation in the case of alfalfa. Thus, for rye and amaranthus correlations of  $0.89 \pm 0.03$  and  $0.95 \pm 0.01$ , respectively, were obtained, while alfalfa at different periods during the same year showed a correlation of  $0.89 \pm 0.01$  and  $0.93 \pm 0.01$ . An equally good correspondence would therefore be expected, at least in the case of amaranthus, between the hourly transpiration rate and the hourly evaporation rate from the shallow tank.

The writers are not, however, urging the merits of the shallow, blackened tank as a means of determining changes in the transpiration coefficient of a plant during the day. The point which it is desired to emphasize is that departures between the hourly transpiration rate and the hourly evaporation rate from any physical system can not be attributed to changes in the transpiration coefficient without first having determined that under less intensive conditions the two systems give graphs which are in accord.



THE ATMOMETER AS A MEANS OF MEASURING SOLAR-RADIATION  
INTENSITY

The simultaneous measurement in the above determinations of the intensity of solar radiation and the evaporation rate from brown and white atmometers affords an opportunity of testing the atmometer as a means of measuring radiation intensity. The radio-atmometer, in which the evaporating surface is darkened so as to absorb as much of the incident radiation as possible, was developed by Livingston (1915, p. 147) primarily for—

measuring the effectiveness of solar radiation as an accelerator of evaporation. It has not been shown to be available for measuring solar radiation as a whole, though its readings no doubt approximate such measurements to a greater or smaller degree.

However, if the increase in evaporation of the radio-atmometer over the white atmometer affords a correct measure of the effect of the intensity of "solar radiation as an accelerator of evaporation" independently of

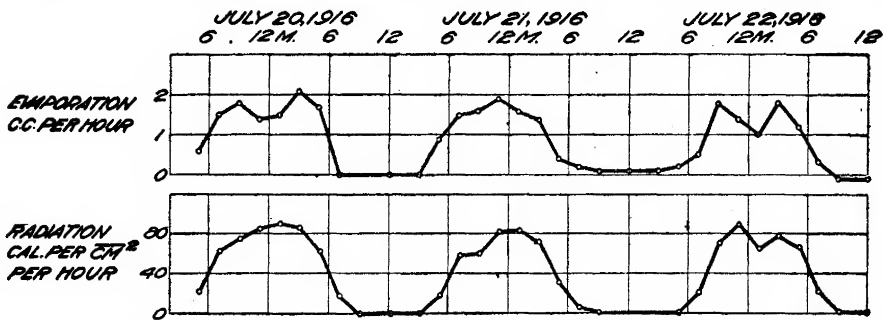


FIG. 4.—A comparison of the hourly radiation with the hourly differences in the evaporation rate from a brown cylindrical radio-atmometer and a white atmometer of the same form.

any peculiarities of the instrument itself, then, conversely, the excess evaporation of the radio-atmometer over the corresponding white type should afford a measure of the intensity of the incident radiation. It is from this latter standpoint that the data are presented. The radio-atmometers employed were of the brown cylindrical type<sup>1</sup> mounted with the cylinders vertical, and in this position do not present a uniform surface area to the march of the sun during the day, as Livingston has already pointed out. Furthermore, the radio-atmometers did not absorb all the incident radiation, the surfaces being brown in color instead of dead black; but, since the coefficient of absorption of a surface does not vary with the amount of energy received, this factor is not of importance in this connection.

The hourly excess of the evaporation from the radio-atmometer over that of the corresponding white cylinders is plotted in figure 4 for the

<sup>1</sup> The new spherical radio-atmometers recently developed by Livingston were not available at the time these measurements were made.

three days during which detailed measurements were made. This hourly excess represents the difference in the hourly values for the brown and white cylinders, as summarized in Table I, which in each instance are based upon the mean of four radio-atmometer observations and the mean of four white-cylinder observations. For comparison, there is also plotted in figure 4 the radiation received on a surface normal to the sun's rays, as measured by a differential telethermograph calibrated by means of an Abbot pyrhelimeter. The integrated radiation—that is, the area inclosed by the graph—for each of the three days investigated, expressed as a percentage of the mean of the three, is as follows: 114, 94, 93. The corresponding excess evaporation of the cylindrical radio-atmometers over the white cylinders for the corresponding three days, expressed also as a percentage of the mean, is 113, 101, 85.

It is of interest to consider in this connection the energy represented by the radiation falling on the cylinder compared with the energy dissipated in the water evaporated. If we consider the mean of all the 11 a. m. to 1 p. m. values for the three days, the mean incident radiation on the radio-atmometer computed from a formula developed by the writers (Briggs and Shantz, 1916a, p. 186) is approximately 1,100 gram-calories per hour, while the average hourly differential evaporation at 11 a. m. and 1 p. m. is 1.5 gms., corresponding to the dissipation of 800 gram-calories per hour. The energy dissipated in the water evaporated thus represents about three-fourths of the total incident energy. The brown cylinder is not a perfect absorbing surface, so that the incident energy would be expected to exceed that dissipated in evaporation, as was found to be the case. It may be possible with the improved spherical atmometers to develop a definite relationship between the incident radiation and the excess evaporation from the black surface, in which event black and white spherical atmometers, when employed together, may be used to measure the intensity of radiation. Black and white Bellani plates may perhaps be used also to measure the vertical component of radiation.

#### SUMMARY

This paper deals with a comparison of the hourly transpiration rate of alfalfa with the hourly evaporation rates from various types of porous-cup atmometers, a filter-paper evaporimeter, a blackened, shallow tank, and a deep tank.

The comparison between the transpiration rate and the evaporation rate was made by superimposing the hourly transpiration graph on each of the hourly evaporation graphs, choosing the scale of ordinates of the transpiration graph so that the total area under the transpiration graph was equal to the total area under the evaporation graph. The average hourly departure of each of the evaporation graphs from the superimposed transpiration graph expressed in percentage of the mean transpiration

for the day was then determined. For the shallow tank the mean hourly departure for the 24-hour period was 17 per cent; for the filter-paper evaporimeter 31 per cent; for the brown cylinder, white sphere, and Bellani plate about 40 per cent; for the white cylinder about 50 per cent; and for the deep tank about 90 per cent of the mean hourly transpiration. The corresponding departures for the daylight hours from 6 a. m. to 6 p. m. were as follows: For the shallow tank, 12 per cent; filter-paper evaporimeter, 22 per cent; brown cylinder, white sphere, and Bellani plate atmometers, about 30 per cent; white cylinder atmometer, 38 per cent; and the deep tank, 93 per cent.

Since the hourly evaporation graphs of the various evaporation systems employed differ widely in form, it does not seem justifiable to attribute the discrepancy between the observed hourly transpiration and that calculated from the evaporation rate of any particular system to a change in the transpiration coefficient of the plant during the day, unless it can be shown that under less extreme conditions the transpiration rate is in accord with the evaporation rate. The plant may not be responding freely to its environment, but a departure in its relative transpiration rate from the evaporation rate of an arbitrarily chosen physical system does not necessarily establish this fact. A close correspondence does not appear to exist between the hourly transpiration rate of normal alfalfa plants and the hourly evaporation rate of any of the systems employed in this investigation. The best agreement in this instance was obtained with the shallow, blackened evaporation tank.

The departure of the hourly evaporation rate of the porous-cup atmometer from the hourly transpiration rate of alfalfa is due largely (1) to the marked increase in the evaporation over transpiration during the night hours; (2) to the marked response of the atmometers to changes in wind velocity, which were not accompanied by corresponding changes in the transpiration rate; and (3) to the lack of a proportionate response on the part of the atmometers to changes in solar radiation.

It should be emphasized in this connection that the failure of an evaporating surface to show a high correlation with the hourly transpiration rate does not necessarily imply a correspondingly low correlation on the daily basis. This is strikingly illustrated by the hourly evaporation rate from the deep tank, which, in these experiments, shows practically no correlation with the hourly transpiration rate, but which on a daily basis was found in 1914 to be correlated with the daily transpiration rate to the extent of  $0.63 \pm 0.01$ .

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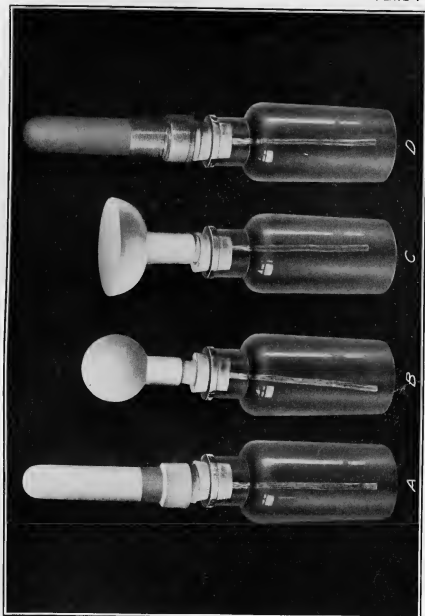
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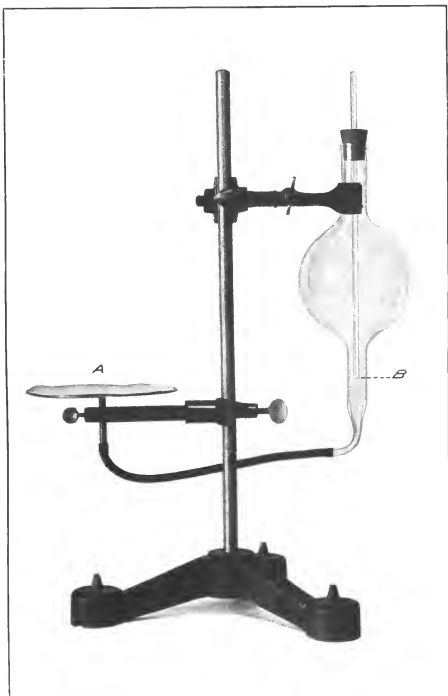
**PLATE 4**

**Types of porous-cup atmometers used in evaporation measurements:**

- A.*—White cylinder.
  - B.*—White sphere.
  - C.*—White Bellani plate.
  - D.*—Brown cylinder.
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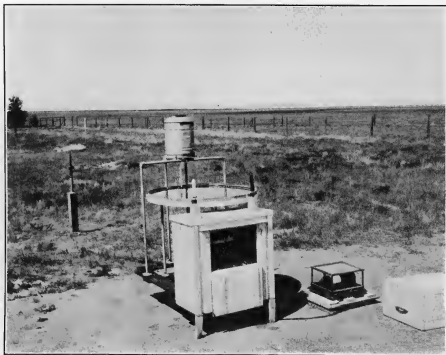
**PLATE 5**

Special filter-paper evaporimeter used by the writers, equipped with Mariotte control (*a*) for the purpose of maintaining a constant water level in the filter-paper container (*b*).

#### PLATE 6

*A.*—Shallow, blackened evaporation tank mounted on automatic balance. The reservoir is shown above in the back. The tank has an area of 6,540 sq. cm., and the water is maintained at a depth of approximately 1 cm.

*B.*—Deep evaporation tank as used at Akron, Colo. This tank is 6 feet in diameter and 2 feet in depth, and is buried in the ground to within 4 inches of the top. The water level is kept at  $4 \pm 1$  inches from the top of the tank. The well of the recording gauge (not shown in the figure) is connected with the tank by a buried pipe. The well for the micrometer gauge may be seen (covered) on the right side of the tank.



A



B



# INFLUENCE OF CROP, SEASON, AND WATER ON THE BACTERIAL ACTIVITIES OF THE SOIL

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## INTRODUCTION

It is of the utmost importance that the quality and quantity of plant food rendered available during the season nicely balance that required by the growing plant, for then we have maximum yield with minimum loss of soil fertility. Most of the changes which take place in the soil constituents are wrought by microorganisms, which bring about the transformation through which nitrogen passes in the soil—that is to say, the transformation from its inert form in the atmosphere to a form available to the growing plant. Furthermore, they play an essential part in the cycles through which hydrogen, sulphur, and carbon pass. Bacteria bring about the mineralization of calcium, iron, phosphorus, and many other inorganic constituents of the plant and animal residues in the soil. Moreover, many of these substances are changed from the insoluble to the soluble form and thus are made available to the growing plant by bacterial activity. At times bacteria have an opposite effect and render many of these substances insoluble, thus preventing in a degree their loss to the growing plant. Or at times they may even compete with the higher plant for the limited supply of nutrient in the soil.

The speed with which these transformations take place within a soil is governed, amongst other factors, by the season of the year, the crop growing upon the soil, and the water which that soil receives. These investigations were undertaken, therefore, with the hope of throwing definite light upon the magnitude of these influences and, furthermore, to correlate the results obtained by the various methods, one with the other, and these in turn with the crop-producing powers of the soil. For these reasons in this work a direct determination was made of the nitric-nitrogen content of the soil as soon as it was taken from the field while other portions of the same samples were used for the determination of the ammonifying and nitrifying powers of the soil. Counts were also made of the number of bacteria developing from the soil on synthetic agar.

A careful review of literature dealing with this phase of the subject has been made, and there is given below a résumé of the most important.

## HISTORICAL REVIEW

## INFLUENCE OF MOISTURE

Long before the process of nitrification was known to be due to micro-organisms, the underlying principles governing the speed of the reaction had been investigated nationally by France, Germany, and Sweden. Among other things, they had learned that there must be a certain proportion of water, and, in order that the maximum yield of nitrates be obtained, that this must be diminished as the soil becomes richer in nitrates. As early as 1887 Dehérain (9) found that the most active nitrification took place when the soil was allowed to become partially dry between the applications of water, and later (12) he found that there was a relationship between the speed of nitrification and the moisture content of fallow soil, the nitrification increasing with the water. Boussingault (63) taught that, when soils contain as much as 60 per cent of water, they lose in a few weeks the greater part of their nitrates. This teaching gave rise to the general belief that denitrification may take place to a great extent in soils, but recent work has amply demonstrated that it is only under extremely abnormal conditions that this becomes an important factor. For this reason literature bearing on this phase of the subject is not considered here.

Dehérain and Demoussy (14) found that the bacterial action of a soil was at its maximum when a rich soil contained 17 per cent of water, but that it decreased if the proportion of water fell to 10 per cent or rose to 25 per cent. With soils less rich in humus a somewhat higher proportion of water was necessary to retard oxidation to any marked degree.

The optimum moisture content for nitrification, according to Dehérain (13), is 25 per cent. An insufficient supply of moisture checked both nitrification and nitrogen fixation. This occurred when the water had been reduced to 16.5 per cent. This, however, would vary with the soil, for Schloesing (50) found bacterial activity less in fine-grained soils than in lighter, coarse-grained soils. In order that nitrification be equally active in both light and heavy soils, the latter must have a higher percentage of water than the former, a difference in moisture content of soil of 1 per cent, according to Dafert and Bollinger (8), being sufficient to produce a marked change in the oxidation going on in the soil.

Frap (16) found that the number of nitrifying organisms in a soil varies with the moisture and that their activity was periodic, rapid nitrification being preceded and followed by periods of less activity. Later he (17) found nitrification to be at its height in soil containing 55.6 per cent of its water capacity. Excessive quantities of water practically stopped nitrification and was much more injurious than too small a quantity. The water requirements, however, varied considerably with the soil.

Coleman's (7) work with a loam soil showed nitrification to be most active when the soil contained 16 per cent of water. It was greatly retarded when the water content was reduced to 10 per cent or increased to 26 per cent. Not only nitrification but ammonification is dependent upon the moisture content of the soil. However, Lipman and Brown (31) found ammonification in a loam soil increased with increased water content even up to 35 per cent of the weight of the soil; but nitrification was most active in the same soil with a moisture content of 15 per cent, was only slightly less active with 10 per cent of moisture, and was still quite marked when the soil contained only 5 per cent of moisture. However, later Lipman, Brown, and Owen (32) found ammonification to increase as the water added increased up to a certain percentage, which varied with the physical nature of the soil; but larger quantities of water reduced the ammonia recovered. Moreover, the work clearly demonstrates that the optimum moisture content for maximum ammonification is higher than it is for maximum nitrification.

Engberding (15) considered that the moisture content of a soil had a greater influence on numbers than did temperature; and the work of King and Doryland (30) clearly indicates that excessive soil moisture reduces both the number and activity of soil bacteria.

Patterson and Scott's (43) work is interesting in that they found nitrification to be inactive in sand and clay soils which still contained about three times as much moisture as in their average air-dry condition. At the lower limits of moisture less water starts nitrification in sand than in clay. At the higher limits of moisture less water stops nitrification in sand than in clay, while the optimum amount of water probably varies for each soil; it is higher for clay, yet for both soils it lies within the range of from 14 to 18 per cent. A rise above the optimum amount of water is more harmful than an equal fall below it.

The work of the Utah Experiment Station (56) demonstrated that the application of irrigation water to a soil has a distinct beneficial effect upon nitrification, being greatest where 15 inches of water were applied when the nitric nitrogen formed amounted to 28.5 pounds per acre-foot of soil. The greatest benefit per inch of water, however, was obtained where only 7.5 inches of water were applied, resulting in 3.8 pounds of nitric nitrogen per inch of water, while where 15 inches of water was applied it was 1.1 pounds of nitric nitrogen per inch of water applied, and when 25 inches of water was applied to the soil the nitric nitrogen produced was only 0.7 pound.

Münter and Robson (4) found that horn meal decomposed more rapidly in dry sandy soil than in clay or loam, while with higher moisture content there was little difference. Ammonium sulphate transformation increased with a higher water content. The best nitrate formation from horn meal occurred in sandy soils. In clay and loam it was best with a

medium water content. Sharp (52) found that the water content most favorable for ammonification was not the optimum condition for nitrification. The former was most rapid with a 25 per cent water content and was not markedly affected by 3 per cent differences. Nitrification was at its maximum when the soil contained 19 per cent of water. When it was increased to 25 per cent, the rate of nitrification was decreased 50 per cent.

McBeth and Smith (38) found a slight variation in the number and nitrifying powers of soil, depending upon the moisture content. However, Gainey (18) considers that among the factors controlling the bacterial activity of a soil the available moisture probably plays a leading part. But we (22) have reported results which indicate that the nitrous nitrogen content of a soil is independent of the irrigation water applied up to 37.5 inches a year. Results recently published from the Utah Experiment Station (21) clearly demonstrate that the influence exerted by water upon ammonifying, nitrifying, and nitrogen-fixing activities of the soil varies greatly with the organic matter in the soil and is much more marked in effect on soils recently manured than on those which have received no manure.

From the literature cited it may be seen that the number of bacteria in a soil and the ammonifying and nitrifying powers of the soil are functions of the moisture content of the soil, and that the optimum varies with the physical and possibly the chemical properties of the soil. But in all soil, to obtain the maximum count and ammonification, the moisture content should be about 6 per cent higher than to obtain maximum nitrification. While the optimum for nitrification varies with different soils, the average would seem to be between 18 and 20 per cent of moisture.

#### INFLUENCE OF CROP

Even as early as 1855 the work at Rothamsted (48) had demonstrated that the beneficial effects of fallowing lies in the increase brought about in the available nitrogen compounds of the soil. Dehérain and Demoussy's (14) work indicated that there is a larger production of nitrates in fallow than in cropped soils, and Pfeiffer (44) considers fallowing an extreme form of soil robbery, for he found that it promotes the activity of the soil organisms and, hence, hastens the exhaustion of the nitrogen supply. But, as it is so clearly pointed out by Warington (63), these results may not hold in a dry climate or during dry seasons; for here bare fallow may not necessitate this loss, and much is to be gained by its practice. But it must always be borne in mind that, if there be sufficient moisture, the loss may be great. For instance, Schneidewind, Meyer, and Münter (51) record a loss in fallow plots of 85.5 pounds per acre, which even exceeded the nitrogen removed by the growing plant on the cropped soil.



On the other hand, McBeth and Smith (38) claim that plots continuously cropped to alfalfa, potatoes, oats, and corn all show a higher nitrifying power than do corresponding fallow plots and that the stimulating effect of crop production on the nitrifying power of a soil is most marked in alfalfa soil. This is in keeping with the recent findings of Vel'bel (60), but is contrary to the findings of many other investigators; for Heinze (24) found fallow to increase the pectin, cellulose, and humus fermenters and also the ammonifiers, nitrifiers, and *Azotobacter*. Russel (48) finds that in late summer fallow land is richer in nitrates than cropped, even after allowing for the nitrogen taken up by the crop; and Heinze shows that repeated cultivation of fallow soil increases the number of organisms in the soil, while Hiltner (25) maintains that no nitrification occurs in soils where legumes are growing vigorously and fixing large quantities of nitrogen. This latter view, however, is the extreme, as is shown by much of the literature on the subject.

Vel'bel and Winkler (61) found that fallow not only increased the assimilable nitrogen but also the available phosphoric acid of the soil and that the increased yield of wheat after fallow is due to these factors. But Bychikhin and Skalski (5) point out that fall fallow is even more wasteful of soil nitrates than is summer fallow, for here the excessive rains wash the soluble nitrates from the soil as fast as formed. The cultivating of fallow further increases the nitrate content, as was shown by Richardson (47). Not only nitrification but all the bacterial activities of a soil are increased by fallow, as may be seen from the following table from the work of the senior author (20) of this paper.

TABLE I.—Average bacterial content and activity of soil with various crops

	Colonies.	Mgm. of NH <sub>3</sub> formed.	Mgm. nitric nitrogen formed.	Mgm. of N fixed.
Virgin soil.....	2. 270	43. 88	2. 09	14. 28
Wheat soil.....	4. 840	52. 94	4. 00	6. 99
Alfalfa soil.....	3. 911	50. 52	2. 25	11. 83
Fallow soil, potato fallow, etc.....	3. 980	83. 42	6. 22	22. 88

The results reported under milligrams of nitrogen fixed indicate that in an arid soil the increased nitrogen fixation in a fallow soil more than offsets the loss of nitrates, even though rapidly formed, for little, if any, would be lost in the drainage waters. These results have recently been confirmed by Reed and Williams (46). Moreover, the number of organisms in the soil and the rapidity of the bacterial activity within the same is going to vary greatly with the thoroughness and time of cultivation, as shown by Dehérain (11), Neish (42), King and Whitson (29), Chester (6), and Quiroga (45), while the number and activity of the organisms in the soil may in a degree determine the speed with which the water evaporates from a soil (57).



The work at the Rothamsted station (63) early demonstrated that the nitrates in the drainage water from the various plots varied greatly, depending upon the crop growing upon the soil, thus indicating a relationship between the available nitrogen in a soil and the crop growing upon the soil; since that time many experimenters have confirmed this conclusion. Furthermore, King and Whitson (28) found 22 per cent more nitrogen developed from soil after clover than from soil after corn, and 13 per cent more than after oats. Later work by them (29) showed that there are greater quantities of nitrates throughout the entire season in soil under corn or potatoes than in soil under clover and oats. Stewart and Greaves (54) found that different plants show a marked difference in their demands upon the nitrate content of the soil, there being a steady decrease in the concentration of the nitrate content of potato and corn lands as the season progressed, while that of fallow and alfalfa remained practically constant, the nitrate content of the latter being uniformly low throughout the season. According to Lyon and Bizzell (33), soil that had produced alfalfa for five years was higher in nitrates than soil that had grown timothy during the same period. Furthermore, the former nitrified ammonium sulphate more readily than did the latter.

Brown (3) found that the rotation of crops caused an increase in number of organisms in a soil, also greater ammonifying, nitrifying, and nitrogen-fixing powers than continuous cropping to either corn or clover. Furthermore, the crop on the soil at time of sampling was of more importance from the bacterial standpoint than the previous crop. However, the preceding crop has a marked effect upon the nitrate content of the soil, as is seen from the work of Lyon and Bizzell (35), where plots that had been planted to certain crops were kept bare of vegetation in the early part of the growing season of 1911. Nitrate determinations of the soil were made and the nitrate present showed a distinct and characteristic relationship to the nitrate content found under the several varieties of plants previously grown upon the soil. Later they (36) showed that alfalfa soil nitrified more rapidly than timothy soil, both in the soil on which the crops had been grown continuously and in that from which they had been removed and the soil kept bare for two seasons. However, one of us (20) has shown that the nitrifying powers of alfalfa soil, while slightly higher than that of virgin soil, is very low when compared with either wheat or potato and fallow soil. Furthermore, the extensive work which has already been published from the Utah Experiment Station (54) demonstrates that there is a very pronounced relationship between the crop growing upon a soil and its nitrate content. However, in this work the nitrate content of the alfalfa and oat soil is very low, while that of corn, potatoes, and fallow is higher. Lyon and Bizzell (37) in 1913 reviewed the work of other investigators and summarized their own work on the influence of higher plants on the formation of nitrates in the soil. From this they conclude that,

aside from the influence of cultivation, the source of greatest difference in the nitrates under various crops may be sought in the inherent differences in the plants of different species in their stimulating and inhibiting influence on the production of nitrates, as well as in their relative rates, amounts, and forms of nitrogen absorbed. Changes in the moisture content and temperature of the soil after early summer had no important effect on the nitrogen content of the soil under plants. On the uncropped soil an increase in moisture content was sometimes accompanied by an increase in nitrates and sometimes by a decrease. But, as pointed out by Kellerman and Wright (27), there may be a variation with different soils. But even the species of organisms found within the soil is controlled to a degree by the crop growing on the soil, as indicated by the work of Stoklasa and Vitek (58).

#### INFLUENCE OF SEASON

The season of the year has a marked influence upon the bacterial activities of the soil, but it is not necessarily correlated with the nitrate content of the soil. Schloesing (50) found the nitrates in the drain water from both manured and unmanured soil high in spring, as compared with midsummer, fall, or winter, thus confirming the results obtained at the Rothamsted station. Shutt (53) reports nearly five times the quantity of nitrates in fallow and cropped soil during June as during November. He does, however, find more during June than during May. The exact season of the year at which the maximum nitrate content is reached will vary with a number of factors, chief amongst which is the kind of crop growing on the soil, for King and Whitson (29) found that the nitrates in the surface foot start in the spring comparatively low and increase rapidly until June 1 on clover and oat ground and until July on corn and potato ground. From these dates they fall more or less rapidly, and the work at the Utah Station (56) demonstrates conclusively that there is a seasonal variation depending upon temperature, crop, and quantity of irrigation water applied to the soil.

Moreover, André (1) has shown that the insoluble nitrogenous compounds of the surface soil are largely transformed into soluble compounds during the summer, and these are widely diffused through the deeper layers of soil during the winter, so that in the spring the lower layers of soil contain more soluble nitrogen than the surface soil. At the end of summer, however, the distribution is quite uniform. This finding has been amply verified by the results reported by Stewart and Greaves (56), Vel'bel (59), Jensen (26), and Lyon and Bizzell (34). These results will vary, however, with different soils, as shown by Russel (49), who reports the fluctuations in nitrates more marked on loams than on clay or sands; moreover, he found the bacterial activities much greater in early summer than later.

Moll (40) even goes so far as to claim from his work that the season of the year is the principal factor in determining the biochemical transformation in a soil, and Heinze (24) found that the number of organisms in a soil was highest in the summer months and lowest in the fall and spring. As already pointed out, the highest nitrifying power of a soil is not necessarily correlated with the highest nitrate content. The latter is highest in spring or early summer, while Vogel (62) found the former to be highest in October and November, after which there was a falling off until April, when it rose again, but not so high as in autumn. This corresponds fairly with the findings of Green (23) for the ammonifying powers of the soil. These findings, however, are contrary to those of Wojtkiewicz (66), who found the maximum number of organisms to occur in soil during the spring and the minimum in the winter. He also notes a correlation between the bacteria present and the amount of nitrates in the soil.

Inasmuch as we have taken no samples while the soil was frozen, no attempt has been made to review the literature dealing with this very interesting phase of the subject.

#### EXPERIMENTAL WORK

##### NATURE OF THE EXPERIMENTAL FIELD

The investigations were conducted on the Greenville farm, belonging to the Utah Experiment Station, which is located 2 miles north of the college farm. The soil of the farm is of a sedimentary nature, being derived from the weathering of the mountain range near by, which consists largely of limestone, quartzite, and dolomite. At the time of Lake Bonneville (19) the mountain streams poured their waters, loaded with the weatherings of these rocks, in the various stages of subdivision (gravel, sand, and silt) into the still waters of the lake. When the swiftly running water of the stream met the quiet water of the lake, the stream began to deposit its load. The gravel and coarser material being deposited first, gave rise to the well-defined deltas found at the mouths of all the larger streams. One of the best defined deltas is that on which the old college farm is located. The fine material, consisting mainly of fine sand, silt, and clay, was carried out farther into the lake, where it was gradually deposited. It is of this sedimentary material that the Greenville farm is composed.

At the beginning of the investigation a soil survey was made of the farm in the following manner: Samples of soil were taken in foot sections from each plot, the corresponding foot sections of these samples were thoroughly mixed and taken to the chemical laboratory, where they were subjected to chemical and physical analyses.

Table II gives the chemical composition of the soil to the depth of 8 feet. The method of analysis followed was that advocated by the Association of Official Agricultural Chemists (65).

TABLE II.—*Chemical composition of the soil of the Greenville (Utah) farm*

Constituent.	Depth of soil.							
	1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	7th foot.	8th foot.
Insoluble residue.....	41.46	35.57	31.65	40.90	28.38	29.22	30.57	30.33
Soluble silica.....	.62	.84	.41	.75	.34	.42	.57	.42
Total.....	42.08	36.41	32.06	41.65	28.72	29.64	31.14	30.75
Potash ( $K_2O$ ).....	.67	.89	.59	.82	.61	.74	.79	.75
Soda ( $Na_2O$ ).....	.35	.47	.47	.62	.37	.42	.45	.74
Lime ( $CaO$ ).....	16.88	17.80	21.34	15.60	22.62	23.15	22.21	21.78
Magnesia ( $MgO$ ).....	6.10	9.46	7.57	7.48	9.36	5.89	6.06	5.63
Iron oxide ( $Fe_2O_3$ )...	3.03	2.69	3.46	2.95	2.17	2.42	2.47	2.54
Alumina ( $Al_2O_3$ ).....	5.64	4.69	3.40	6.09	5.33	8.07	7.90	9.03
Phosphoric acid ( $P_2O_5$ ).....	.41	.29	.34	.19	.12	.06	.07	.11
Carbon dioxide ( $CO_2$ )..	19.83	23.11	26.67	20.88	29.31	29.57	28.80	28.13
Volatile matter.....	5.60	3.38	3.93	4.23	.91	.95	.....	.24
Total.....	100.69	99.29	99.93	100.51	99.52	100.91	99.92	99.68
Humus.....	.53	1.00	.61	.47	1.13	.60	.44	.57
Nitrogen.....	.139	.117	.080	.175	.072	.070	.062	.066

An examination of Table II will show that we have here a soil, like all of our Utah soils, exceptionally rich in the essential plant foods. The potassium is equally as high in the eighth and intermediate feet as in the first foot. The phosphoric acid is high in the first foot but gradually decreases in each succeeding foot. The humus and nitrogen, as is characteristic of the soils of arid America, are low. One of the most important considerations, however, from the viewpoint of this investigation, is the fact that the calcium and magnesium carbonate content of the soil is exceptionally high. In fact, the results indicate that 43 per cent of the surface foot of soil is calcium and magnesium carbonate and that the amount increases with depth to the fifth foot, after which the magnesium content is practically the same as in the first foot, while the calcium carbonate also increases with depth to a maximum in the fifth foot and then remains practically constant.

From the work of previous investigators on the magnesia content of soils one would conclude that the soil would be sterile, but just the contrary is true—the soil is remarkably fertile and produces excellent crops even without the addition of barnyard manure. With the single exception of its low humus content, the soil is ideally adapted both chemically and bacteriologically to support rapid bacterial action.

Table III gives the physical composition of the soil of the Greenville farm. The results show the soil to be a good loam of remarkable uniformity throughout the 8 feet.



TABLE III.—*Physical analysis of the soil of the Greenville (Utah) farm*

Item.	Depth of soil.							
	1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	7th foot.	8th foot.
Coarse sand.....	0.21	0.17	0.68	1.02	0.09	0.34	0.47	0.09
Medium sand.....	9.63	8.29	6.63	9.63	9.53	9.48	8.91	7.08
Fine sand.....	30.04	32.54	29.49	33.06	36.92	33.79	35.34	34.25
Coarse silt.....	32.25	32.81	32.62	28.51	28.65	30.49	31.65	32.65
Medium silt.....	12.30	10.46	10.89	10.95	10.46	10.85	9.92	9.89
Fine silt.....	6.25	4.81	7.27	6.94	4.85	5.86	5.56	5.84
Clay.....	7.02	7.12	10.13	7.52	7.82	6.78	6.52	7.57
Moisture.....	1.60	1.47	1.13	1.49	.95	1.01	1.01	.84
Soluble and lost.....	.10	2.33	1.16	.83	.73	1.40	1.42	1.99
Specific gravity.....	2.67	2.72	2.80	2.69	2.76	2.79	2.71	2.76
Apparent sp. gr.....	1.23	1.27	1.30	1.29	1.33	1.34	1.39	1.35
Water-soluble salts...	.06	.11	.14	.16	.08	.09	.15	.09

## PLAN OF EXPERIMENT

The experimental field was divided into 20 plots one-twenty-sixth of an acre in area. Each plot was leveled and banked up around edges, so that the water applied would distribute itself equally over the entire area of the plot.

Leading to each series of plots were wooden lateral flumes, so arranged that the measured water could be accurately applied. The plan of the field and the distribution of the laterals are shown in figure 1.

The field was divided into five equal sets of plots. The first set was left fallow, the second was planted to alfalfa, the third was planted to corn, the fourth was planted to potatoes, and the fifth was planted to oats. One of these sets received a maximum, one a medium, one a minimum application of water, and one set was unirrigated. The plots were sampled during the spring (about the middle of April), midsummer (about the last of July), and in the fall (the last of October or the first of November). The samples were analyzed for moisture, nitric nitrogen, number of bacteria developing on synthetic media, and the ammonifying and nitrifying powers. The irrigation and sampling were so arranged that results from the cropped irrigated plots could be compared with the unirrigated plot of the same series and also with the fallow plots receiving a corresponding amount of irrigation water.

The arrangement of the plots, crop growing upon each, and amount of water applied during the last nine years are indicated below:

## (a) ALFALFA:

- Plot 31G, 37.5 inches of water applied in five equal irrigations.
- Plot 32G, 25 inches of water applied in five equal irrigations.
- Plot 33G, 15 inches of water applied in five equal irrigations.
- Plot 34G, unirrigated.

## (b) POTATOES:

- Plot 35G, 37.5 inches of water applied in five equal irrigations.
- Plot 36G, 25 inches of water applied in five equal irrigations.
- Plot 37G, 15 inches of water applied in five equal irrigations.
- Plot 38G, unirrigated.



## (c) FALLOW:

Plot 39G, 37.5 inches of water applied in five equal irrigations.

Plot 40G, 25 inches of water applied in five equal irrigations.

Plot 41G, 15 inches of water applied in five equal irrigations.

Plot 42G, unirrigated.

## (d) OATS:

Plot 43G, 37.5 inches of water applied in five equal irrigations.

Plot 44G, 25 inches of water applied in five equal irrigations.

Plot 45G, 15 inches of water applied in five equal irrigations.

Plot 46G, unirrigated.

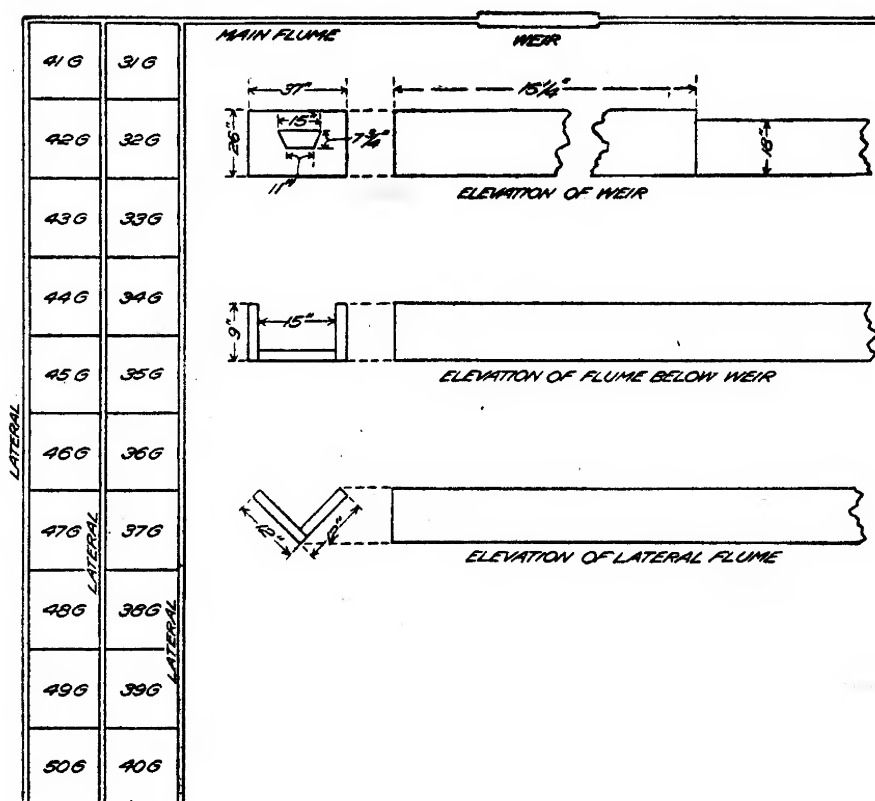


FIG. 1.—Plan of plots 30 G to 50 G, Greenville farm, showing main flume and laterals.

## (e) CORN:

Plot 47G, 37.5 inches of water applied in five equal irrigations.

Plot 48G, 25 inches of water applied in five equal irrigations.

Plot 49G, 15 inches of water applied in five equal irrigations.

Plot 50G, unirrigated.

In the work it was desired to eliminate, as far as possible, all variable factors except crop and irrigation water. For this reason the depth of plowing, time of planting, cultivation, etc. were as nearly uniform as possible on all of the plots.

## METHOD OF SAMPLING

For the determination of nitric nitrogen samples of soil were taken in foot sections to a depth of 6 feet, by means of a King soil tube. Single samples were taken from as near the center of the plot as possible, care being taken that separate borings were at least 3 feet apart. The samples thus obtained were taken to the chemical laboratory, where nitric nitrogen and moisture determinations were made immediately upon the samples. The results reported here, therefore, are all referred to moisture-free basis.

For the bacteriological work all possible precautions were taken, when collecting the samples, against the contamination of one sample by another. The surface soil to a depth of half an inch was scraped off by means of a sterile spade. A hole 12 inches deep was dug, and a slice of soil to this depth was taken from the side of the hole and placed in a sterile mixing pan. This process was repeated from four or five places in the field, and then the contents of the pan was carefully mixed by means of a sterile spatula. From this composite sample a representative portion, about 5 pounds of soil, was placed in a sterile ore sack and conveyed to the laboratory for analysis.

Before each sampling, the spade, mixing pan, and spatula were all carefully sterilized by heat from a plumber's torch, thus preventing the transfer of organisms from one soil to another. The samples were immediately transferred to the laboratory, partly air-dried in the dark, and then ground in a sterile mortar, all coarse rock being removed. The analysis was begun in all cases within 24 hours of the time of taking samples.

## METHODS OF ANALYSIS

The soil extract for the determination of nitric nitrogen was obtained by means of the Pasteur-Chamberland filter. For rapid work a series of 24 Pasteur-Chamberland filters was arranged together and connected to a tank of compressed air filled by means of an air pump run by a  $\frac{1}{2}$  horsepower electric motor. Fifty gm. portions of the soil were triturated in a mortar with 250 c. c. of distilled water, and 2 gm. of quicklime for 2 minutes, allowed to settle 20 minutes, and then filtered through the Pasteur-Chamberland filter. By this method a clear, colorless filtrate was readily obtained.

An aliquot portion (50 c. c.) was immediately measured into 100-c. c. beakers and evaporated to dryness on the electric hot plate. The residue was treated with 1 c. c. of phenol-disulphonic acid equally distributed over it, and then allowed to stand for 10 minutes. This solution was diluted with water and the excess of acid neutralized with dilute ammonia. The color produced was compared with that produced by a standard solution of potassium nitrate treated in the same manner. The quantity

of chlorids in the soil solution was not sufficient to affect the sensitiveness of the method (55).

The number of organisms was determined by growing them on a modified synthetic agar having the following composition:

- 1,000 c. c. of distilled water.
- 10 gm. of dextrose.
- 0.5 gm. of dipotassium phosphate ( $K_2HPO_4$ ).
- 0.2 gm. of magnesium sulphate ( $MgSO_4$ ).
- 2 gm. of powdered agar per 100 c. c. of media.

After the samples of soil had been carefully mixed by shaking, 100 gm. were weighed on a sterile watch glass, using a small sterile spatula. This soil was transferred to 200 c. c. of sterile water and shaken for one minute, 1 c. c. of this suspension transferred to 99 c. c. of sterile water, and the dilution continued with 9 c. c. of sterile water. The plates were made so as to give a dilution of 1 to 20,000 and 1 to 200,000. They were incubated at 28° C. for four days and then counted. No attempt was made to differentiate between bacteria and molds, but all were listed together as total numbers of colonies.

The ammonifying power of the soil was determined by weighing 100-gm. portions of the soil and 2 gm. of dried blood into sterile tumblers and covering them with petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to 18 per cent with sterile water. The samples were incubated at 28° to 30° C. for four days and the ammonia determined by transferring to Kjeldahl flasks with 250 c. c. of distilled water, adding 2 gm. of magnesium oxid and distilling into *N/10* sulphuric acid. The determinations were all made in duplicates and compared with sterile blanks.

The nitrifying power of the soils was determined in tumblers like the ammonifying power, except that they were incubated for 21 days. The moisture content was made up weekly to the initial 18 per cent.

At the end of the incubation period each soil was transferred with 250 c. c. of distilled water to a 1-pint Mason fruit jar. Two gm. of powdered lime were added and the jar placed in the shaking machine for 10 minutes, after which it stood in the closed jar until clear. This never required over two hours. At the end of this time an aliquot part (100 c. c.) was measured into a flask and the nitrates determined by the aluminum reduction method (4).

The soil on which the experiments were conducted is extremely fertile, as is shown by the fact that the soil has been cropped for 48 years without the addition of barnyard manure or commercial fertilizers, yet during the last 4 years it has yielded fair crops. This is shown in Table IV, which gives the average yearly yield in pounds per acre. From these yields and the average percentage of nitrogen in the crops under similar irrigated conditions (64) the average amount of nitrogen removed per year has been calculated.

TABLE IV.—Average yield of dry matter and nitrogen from the experimental plots on Greenville farm

[Expressed as pounds per acre]

Water applied.	Alfalfa.			Potatoes.		
	Plot No.	Hay.	Nitrogen.	Plot No.	Tubers.	Nitrogen.
<i>Inches.</i>						
37.5.....	31	10,464	282.5	35	1,464	20.4
25.0.....	32	9,963	265.0	36	1,540	24.8
15.0.....	33	9,779	259.1	37	1,759	33.2
None.....	34	6,808	170.1	38	1,075	19.1

Water applied.	Oats.				Corn.			
	Plot No.	Grain.	Straw.	Nitrogen.	Plot No.	Grain.	Stover.	Nitrogen.
<i>Inches.</i>								
37.5.....	43	2,273	2,989	89.5	47	2,080	3,316	66.3
25.0.....	44	2,093	2,581	83.9	48	1,995	3,332	69.6
15.0.....	45	1,885	1,821	71.7	49	2,179	3,605	76.6
None.....	46	1,560	1,928	64.0	50	1,600	3,280	62.3

## INFLUENCE OF WATER ON THE NITRIC NITROGEN OF THE SOIL

The results obtained by a direct determination of the nitric nitrogen of the soil have been so arranged that the plots growing each specific crop but receiving different quantities of irrigation water are placed in the same table. This makes it possible to directly compare the plots receiving varying quantities—37.5, 25, and 15 inches of irrigation water—with each other and each of these in turn with the unirrigated plot. The results are reported as pounds per acre and are the average for three years. The plots were sampled in the spring (May 1), midsummer (August 1), and in the fall (November 28).

## I.—ALFALFA LAND

There were four plots in this series, one receiving 37.5 inches of irrigation water, one 25 inches, one 15 inches, and one was unirrigated. The average results expressed as pounds per acre are given in Table V.

During the spring and summer the nitric nitrogen is about uniformly distributed throughout the 6 feet of soil, but in the fall it has become concentrated in the surface 2 feet. The difference in the nitric-nitrogen content of the soil of the various plots between fall and spring is quite significant, for the plots which had received no irrigation water throughout the season are richer in nitric nitrogen in the spring than in the fall, while all of the irrigated plots are much richer in the fall than in the spring. This is likely due to the moist conditions of the irrigated plots in the fall, as the winter rains would carry the soluble constituents of

these plots to a greater depth than they would in the unirrigated. This conclusion is borne out by the results previously published by us (56). In this earlier work we took samples to a depth of 10 feet and found that there is no loss of nitric nitrogen from these plots during the winter months, thus showing that the nitric nitrogen is carried below the 6-foot level.

TABLE V.—*Nitric nitrogen in alfalfa land—Average for three years*

[Results expressed as pounds per acre]

Plot No.	Period.	Water applied in five applications.	Depth of soil.						Total.
			1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
		<i>Inches.</i>							
31.....	Spring.....	37.5	3.6	1.9	0.6	2.0	1.4	1.0	10.5
32.....	do.....	25.0	1.9	2.0	1.8	1.4	2.5	1.4	11.0
33.....	do.....	15.0	3.4	.9	11.6	3.3	1.6	1.9	22.7
34.....	do.....	None.	10.9	5.1	11.4	2.2	9.3	6.0	44.9
31.....	Summer...	37.5	7.1	2.5	2.5	1.9	2.1	.4	16.5
32.....	do.....	25.0	2.4	3.2	2.3	1.8	1.4	1.5	12.6
33.....	do.....	15.0	5.4	.8	2.1	1.7	3.1	1.4	15.1
34.....	do.....	None.	3.3	8.8	2.2	1.4	2.6	1.0	19.3
31.....	Fall.....	37.5	8.5	4.2	2.4	3.5	2.8	2.0	23.4
32.....	do.....	25.0	14.9	9.0	3.2	2.4	2.0	4.4	35.9
33.....	do.....	15.0	10.7	10.4	4.4	2.3	2.9	1.8	32.5
34.....	do.....	None.	19.5	9.7	6.5	3.0	2.1	1.1	41.9

The results at first glance might be taken to indicate that the application of irrigation water to this soil has retarded nitrification, but it must be borne in mind that the nitrogen removed from the irrigated plots was much greater than that removed from the unirrigated plot. This nitrogen must come either directly from the air through the intervention of lower organisms or from the soluble nitrates of the soil. The evidence is practically conclusive that the alfalfa plant feeds upon the latter as long as it is available and only turns to the atmospheric nitrogen when the soil supply has been reduced to a certain low minimum.

The quantity of nitric nitrogen in the soil decreases as the quantity of water applied increases, and this is much more pronounced in the spring than it is in the summer or fall. But the influence of the irrigation water is quite noticeable throughout the year. The results as a whole clearly indicate that the nitric nitrogen of the alfalfa soil is low throughout the season, in spite of the fact that the alfalfa is capable of indirectly drawing upon the atmospheric nitrogen. The nitrogen found within the alfalfa removed from these plots comes from a number of sources—the atmospheric nitrogen, the nitrogen contained within the 6 feet of soil, and nitrogen from a depth greater than 6 feet. A comparison of these results with those reported where the soil was sampled to a depth of 10 feet makes the conclusion practically certain that much nitric nitrogen



has been brought up from below 6 feet. It is also interesting to note that, while the total nitrogen disappearing from each plot bears a relationship to the water applied, the quantity per inch of water is greatest where only 15 inches of water were applied. In fact, it is twice as great as where 37.5 inches of water are applied to the soil (Table VI).

TABLE VI.—*Summary of nitrogen transformations in alfalfa soil*

[Results expressed in pounds]

Character of nitrogen.	Water applied.			
	37.5 inches.	25 inches.	15 inches.	None.
Nitrogen removed in crop.....	282.5	265	259.1	170.1
Nitrogen in soil in spring.....	10.5	11	22.7	44.9
Nitrogen in soil in fall.....	23.4	35.9	32.5	41.9
Original soil nitrogen removed.....	-12.9	-24.9	-9.8	3.0
Nitrogen formed during season.....	295.4	289.9	268.9	167.1
Excess of nitrogen formed during season in the irrigated plots.....	128.3	122.8	101.8	.....
Excess per acre-inch of water.....	3.4	4.9	6.8	.....

## 2.—POTATO LAND

The plots in the series on potato land were four in number. One received 37.5 inches, one 25 inches, one 15 inches of irrigation water, while one was unirrigated. All other conditions of the plots were kept as nearly uniform as possible, so that any difference which is found to exist in the nitric nitrogen must be due to the variable factor—the water applied. The average results for the three years are given in Table VII.

TABLE VII.—*Nitric nitrogen in potato land—Average for three years*

[Results expressed as pounds per acre]

Plot No.	Period.	Water applied in five applications.	Depth of soil.						Total.
			1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
		<i>Inches.</i>							
35.....	Spring.....	37.5	11.4	12.2	2.3	2.7	2.2	2.9	33.7
36.....	...do.....	25.0	17.1	8.2	8.9	7.9	5.0	2.8	49.9
37.....	...do.....	15.0	20.5	8.7	4.0	4.0	3.1	4.0	44.3
38.....	...do.....	None.	10.9	29.0	43.6	46.4	38.5	27.9	196.3
35.....	Summer...	37.5	2.6	2.0	12.8	7.9	8.4	4.3	38.0
36.....	...do.....	25.0	9.8	4.4	8.9	6.3	6.7	8.3	44.4
37.....	...do.....	15.0	16.1	15.2	7.2	3.2	3.0	2.4	47.1
38.....	...do.....	None.	25.9	8.9	17.5	25.1	18.4	17.9	113.7
35.....	Fall.....	37.5	9.9	6.1	3.7	4.9	3.0	3.9	31.5
36.....	...do.....	25.0	18.9	5.5	6.2	7.9	5.2	4.2	47.9
37.....	...do.....	15.0	10.9	3.7	2.5	6.6	7.2	4.4	35.3
38.....	...do.....	None.	34.6	17.6	9.8	11.5	19.1	9.6	102.2

The nitric nitrogen in the surface foot of all these plots is very high during the spring and fall. In the summer they are high only in the plots which received 15 inches and no irrigation water, thus showing the effect of the water upon the soluble nitrates of the soil. In all of the irrigated plots the total nitric nitrogen found in the soil at the end of the season is practically the same as in the spring. But the unirrigated plot, between the fall and spring sampling, gains 94 pounds of nitric nitrogen. Now, if this be a correct measure of the nitric nitrogen produced by the various plots during the winter and spring months, there must have been large quantities of the nitric nitrogen which was produced in the irrigated soil carried below 6 feet. It is not likely that the water content of the irrigated plots would have become sufficient to retard nitrification to this extent, although it may have been retarded to a degree by the lower temperature which would prevail in the heavily irrigated plots. But this would be far from sufficient to account for the excess quantity of nitrates found in the unirrigated soil. Much of this, therefore, must have disappeared in the drain waters.

The influence of the irrigation water upon the nitric-nitrogen content of the soil is noticeable throughout the year, and the quantity present at any time decreases as the proportion of water applied to the soil increases. It is interesting to compare the total nitric nitrogen of the various plots in spring and fall with that removed by the crop under the various treatments (Table VIII).

TABLE VIII.—*Summary of nitrogen transformations in potato land*

[Results expressed in pounds]

Character of nitrogen.	Water applied.			
	37.5 inches.	25 inches.	15 inches.	None.
Nitrogen removed in crop.....	20.4	24.8	33.2	19.1
Nitric nitrogen in soil in spring.....	33.7	49.9	44.3	196.3
Nitric nitrogen in soil in fall.....	31.5	47.9	35.3	102.2
Original nitric nitrogen removed from soil.....	2.2	2.0	9.0	94.1
Nitric nitrogen formed during season.....	18.2	22.8	24.2	-75.0
Excess of nitric nitrogen formed during season in irrigated soil.....	93.2	97.8	99.2	.....
Excess per acre-inch of water applied.....	2.5	3.9	6.6	.....

This gives the best results both for total quantity of water applied and quantity per inch of water where an application of 15 inches of water is used. Furthermore, these results indicate that one of two things must have occurred in these plots: Either the formation of nitric nitrogen has been increased to a greater extent than shown by these results by the irrigation water, or else the water has carried none to a greater depth than 6 feet. And all of our results have pointed strongly

to the conclusion that this latter assumption is not the correct one. Hence, the quantity which has been formed, due to the irrigation water, must be larger than is here indicated. The large quantity of nitric nitrogen which has disappeared from the unirrigated plot during the summer could not have been due to denitrification, for there was nothing in the conditions of this plot which would favor the denitrification, the quantity of organic matter present was low, and the aeration would be better than in the irrigated soil. The only conditions which could in any way favor it are larger quantities of nitrates present, and these may favor the rapid growth of other organisms. Furthermore, our results show the existence of many more organisms in this unirrigated plot during the spring than there are in any of the other plots on which potatoes were grown. Hence, the most reasonable explanation is that the nitrates disappeared because the bacterial flora of the soil had transformed them into proteins in their metabolic processes.

### 3.—OAT LAND

There were four plots in this series and the water applied and method of application were the same as in the previous series. The average results for the three years are given in Table IX.

TABLE IX.—*Nitric nitrogen in oat land—Average for three years*

[Results expressed as pounds per acre]

Plot No.	Period.	Water applied in five applications.	Depth of soil.						Total.
			1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
		<i>Inches.</i>							
43.....	Spring.....	37.5	13.7	4.3	5.9	1.8	3.4	2.6	31.7
44.....	...do.....	25.0	15.8	4.9	3.4	5.3	1.8	4.3	35.5
45.....	...do.....	15.0	7.6	6.9	9.6	4.6	1.7	3.0	33.4
46.....	...do.....	None.	9.1	5.9	4.0	6.1	10.7	6.3	42.1
43.....	Summer...	37.5	2.2	2.0	3.2	1.2	1.9	1.8	12.1
44.....	...do.....	25.0	2.6	1.7	3.2	2.8	2.4	3.3	16.0
45.....	...do.....	15.0	5.1	3.8	3.7	1.7	3.0	.8	18.1
46.....	...do.....	None.	3.3	1.6	1.5	1.6	1.6	.8	10.4
43.....	Fall.....	37.5	5.6	3.0	4.0	2.8	2.5	2.5	20.4
44.....	...do.....	25.0	4.0	3.2	3.0	2.7	2.8	2.6	18.3
45.....	...do.....	15.0	4.2	3.3	4.1	3.4	2.9	2.7	20.6
46.....	...do.....	None.	6.7	3.9	3.7	3.3	2.9	2.8	23.3

The nitric nitrogen in all of the oat plots is quite uniform during the spring, but by midsummer the large accumulations of the surface foot have disappeared. This may be due to the leaching out of the nitrates by the irrigation water, or the rapidly growing plant may have utilized it. Very likely it is due to the latter factor, for the loss is nearly uniform from the irrigated and unirrigated plots. The only change which

we note in the fall is an accumulation of more nitrates in the surface feet. It is interesting to note that each plot gains considerable nitric nitrogen during the winter months. The gain is least in the plot which received 37.5 inches of water, but is quite uniform in each of the others.

The summarized results of the nitrogen removed in the crop, together with the original nitric nitrogen present in the soil and the amount formed during the irrigation season, are recorded in Table X.

TABLE X.—*Summary of nitrogen transformations in oat land*

[Results expressed in pounds]

Character of nitrogen.	Water applied.			
	37.5 inches.	25 inches.	15 inches.	None.
Nitrogen removed in crop.....	89.5	83.9	71.7	64.0
Nitrogen in soil in spring.....	31.7	35.5	33.4	42.1
Nitrogen in soil in fall.....	20.4	18.3	20.6	23.3
Original soil nitrogen removed.....	11.3	17.2	12.8	18.8
Nitrogen formed during season.....	78.2	66.7	58.9	45.2
Excess of nitric nitrogen in irrigated plots.....	33.0	21.5	13.7	.....
Excess per acre-inch of water.....	.88	.86	.91	.....

From this it may be seen that the greatest quantity of nitric nitrogen was produced in the plot which received the greatest quantity of water, but the amount per acre-inch of water is slightly higher in the plot which received only 15 inches of water. The actual difference in the quantity of nitrogen produced in the various plots would be even greater than the figures herein represent them to be, for the tendency would be for the larger quantities of water to carry some of the nitric nitrogen below the sixth foot. It is important to note that the order of effectiveness was found by us to be practically the same as here reported when we sampled the plots to a depth of 10 feet; yet in this work they are more regular than in previously reported results.

#### 4.—CORN LAND

There were four plots in this series. One received 37.5 inches of water, one 25 inches, one 15 inches, and one was unirrigated. With the exception of the amounts of water applied, the plots were all uniformly handled throughout the experiment. The average results for the three years are given in Table XI.

The nitric nitrogen is high in the surface foot of all the plots in the spring. This is especially the case where only 15 inches or no irrigation water was applied, but in the summer it becomes very low. This is due mainly to the removal of the nitric nitrogen by the rapidly growing plant. The water, however, does play some part, for we find that the nitric nitrogen of the surface foot disappears more rapidly from the

irrigated plots than from the unirrigated plot. This difference is very marked if we compare the results obtained on the plot receiving 37.5 inches of water, with the plot receiving no water for summer and fall. The quantity found in the surface foot of the unirrigated plot is about the same in the fall as in the spring, while the irrigated contains less than half as much.

TABLE XI.—*Nitric nitrogen in corn land—Average for three years*

[Results expressed as pounds per acre]

Plot No.	Period.	Water applied (five applications).	Depth of soil.						Total.
			1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
		<i>Inches.</i>							
47.....	Spring....	37.5	9.1	3.4	3.3	2.1	1.7	4.2	23.8
48.....	do.....	25.0	7.2	2.3	2.0	1.9	1.9	2.2	17.5
49.....	do.....	15.0	12.3	8.5	4.1	2.0	1.6	1.9	30.4
50.....	do.....	None.	12.0	4.1	1.9	2.5	3.4	3.5	27.4
47.....	Summer....	27.5	1.2	2.9	8.5	4.1	4.0	1.4	22.1
48.....	do.....	25.0	1.7	1.6	5.5	3.9	1.9	2.2	16.8
49.....	do.....	15.0	1.4	5.2	3.7	2.5	3.2	3.3	19.3
50.....	do.....	None.	3.3	3.1	2.9	2.6	3.2	2.2	17.3
47.....	Fall.....	37.5	3.0	3.6	2.7	2.0	2.7	2.4	16.4
48.....	do.....	25.0	3.7	3.0	2.2	2.7	2.4	3.2	17.2
49.....	do.....	15.0	5.8	2.5	3.2	3.0	3.2	3.2	20.9
50.....	do.....	None.	11.2	4.6	3.1	3.5	5.0	5.9	33.3

The total quantity found in the various plots in the spring is nearly the same as that found in them during the fall. A summarized inventory of the nitrogen removed by the crop, together with that formed in the various plots, is given in Table XII. Here we note a decrease in the nitrogen removed in the crop, as the water applied increases above 15 inches. This can be accounted for by the larger quantities of water washing the nitric nitrogen beyond the sphere of action of the roots, thus making the nitrogen the limiting factor.

TABLE XII.—*Summary of nitrogen transformations in corn land*

[Results expressed in pounds of nitric nitrogen per acre-inch]

Character of nitrogen.	Water applied.			
	37.5 inches.	25 inches.	15 inches.	None.
Nitrogen removed in crop.....	66.3	69.6	76.6	62.3
Nitrogen in soil in spring.....	23.8	17.5	30.4	27.4
Nitrogen in soil in fall.....	16.4	17.2	20.9	33.3
Original soil nitrogen removed.....	7.4	.3	9.5	— 5.9
Nitrogen formed during season.....	58.9	69.3	67.1	68.2



These results would lead us to believe that the quantity of nitric nitrogen formed during the season was much less where 37.5 inches of water were applied than where 25 inches, 15 inches, and no water was used. This is probably not correct, but in this plot much nitric nitrogen has been removed in the drain waters. This set of plots differs markedly from all others, in that no more nitric nitrogen is accounted for in the irrigated than in the nonirrigated soil. Nor do these results correspond with those previously published by us; however, the variation may be due to the difference in depth of sampling, for in previously reported results we have sampled to a depth of 10 feet, while here we have sampled to a depth of only 6. In this latter case, apparently, the total herein reported does not represent all the nitric nitrogen formed within the soil; but during the summer, where the heavier applications of irrigation water are made, much of the nitric nitrogen is carried below 6 feet.

## 5.—FALLOW SOIL

Four plots were kept fallow throughout the experiment. One of these received 37.5 inches of water, one 25 inches, one 15 inches, while one was unirrigated. With the exception of water applied, the plots were all treated the same. The summarized results for the three years, reported as pounds per acre, are given in Table XIII.

TABLE XIII.—*Nitric nitrogen in fallow land—average for three years*

[Results expressed as pounds per acre]

Plot No.	Period.	Water applied (five applications).	Depth of soil.						Total.
			1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
		<i>Inches.</i>							
39.....	Spring....	37.5	15.6	14.5	10.7	5.5	7.2	5.9	59.4
40.....	...do.....	25.0	10.1	6.0	4.8	5.5	10.5	19.4	56.3
41.....	...do.....	15.0	10.4	15.6	9.2	9.1	13.6	9.5	67.4
42.....	...do.....	None.	18.9	39.6	31.9	14.8	21.7	16.1	143.0
39.....	Summer...	37.5	4.8	6.9	5.3	9.4	8.6	7.2	42.2
40.....	...do.....	25.0	3.5	13.4	13.1	15.2	19.6	7.9	72.7
41.....	...do.....	15.0	8.4	6.2	14.4	2.6	2.1	3.1	26.8
42.....	...do.....	None.	15.5	9.7	8.2	9.7	13.6	16.1	72.8
39.....	Fall.....	37.5	9.4	7.2	7.8	9.0	6.3	5.3	45.0
40.....	...do.....	25.0	16.5	7.9	9.7	6.0	7.8	4.5	52.4
41.....	...do.....	15.0	10.1	7.4	6.3	7.2	7.7	7.3	46.0
42.....	...do.....	None.	31.5	17.6	18.1	15.9	11.5	12.3	106.9

The quantity of nitric nitrogen in the surface foot of all the plots is high in the spring but greatly decreases in the surface foot of all the irrigated plots in the summer. There is, however, a reconcentration of the nitric nitrogen in the surface during the fall. This is very pronounced in the case of the plot which did not receive irrigation water. The total quantity of nitric nitrogen in each specific plot is higher in the spring

than in the fall, thus showing a gain for the winter months and a loss for the summer months. This loss can not be due to the water applied, because it is most pronounced in the unirrigated plots and probably represents the quantity transferred into complex proteins by the bacteria. Taken as a whole, the results would tend to indicate that the irrigation water had decreased, instead of accelerating the formation of nitric nitrogen in these plots. When we take the average total amount of nitric nitrogen in all cropped and fallow plots receiving the same amount of irrigation water, we note a pronounced difference in the quantity of nitric nitrogen in the plots receiving varying quantities of water. These averages are given in Table XIV.

TABLE XIV.—Average quantity of nitric nitrogen found in the plots receiving the various amounts of irrigation water

[Results expressed as pounds]

Water applied (inches).	Spring.	Summer.	Fall.	Average.
37.5.....	31.8	26.2	27.3	28.4
25.0.....	34.0	32.5	34.3	33.6
15.0.....	39.6	25.3	31.1	32.0
None.....	90.7	46.7	61.5	66.3

The quantity of nitric nitrogen found within the surface 6 feet of soil decreases as the water applied increases. The difference is very noticeable in all of the plots in the spring. During the summer and fall all irrigated plots tend to reach a similar concentration of nitric nitrogen. But even then there is twice as much in the unirrigated as in the irrigated, and during the spring there is three times as much in the nonirrigated as in the plot receiving 37.5 inches of water.

In previously reported work (56) we have shown that the application of irrigation water increases the quantity of nitric nitrogen actually formed within the soil; hence, the difference between that found within the irrigated and the unirrigated soils must represent the quantity removed by the crop plus that washed to a region below 6 feet in the soil. These results prove conclusively that this is no small quantity, especially when large quantities of water are applied to the soil. Hence, the excessive use of irrigation water is not only a waste but it decreases the yield upon a given soil; and this latter effect is due in all probability to the rapid removal of the soluble nitrates beyond the reach of the growing plant.

#### INFLUENCE OF THE CROP ON THE NITROGEN OF THE SOIL

The experiment was so planned that it would also give information on the influence of crop upon the nitric nitrogen of the soil. Therefore, the results have been rearranged so as to compare plots receiving the same quantity of water but growing different crops. In these the variable is the crop, while the water remains a constant.

## PLOTS RECEIVING 37.5 INCHES OF IRRIGATION WATER

In this series we have alfalfa, potato, oat, corn, and fallow plots, each receiving 37.5 inches of irrigation water, so that any difference noted in the nitric nitrogen of the soil must be due either directly or indirectly to the crop growing upon the same. The summarized results for the three years are given in Table XV.

TABLE XV.—*Nitric nitrogen in soil growing various crops and receiving 37.5 inches of irrigation water—Average for three years*

[Results expressed as pounds per acre]

Period.	Crop.	Depth of soil.						Total.
		1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
Spring.....	Alfalfa.....	3.6	1.9	0.6	2.0	1.4	1.0	10.5
Do.....	Oats.....	13.7	4.3	5.9	1.8	3.4	2.6	31.7
Do.....	Corn.....	9.1	3.4	3.3	2.1	1.7	4.2	23.8
Do.....	Potatoes.....	11.4	12.2	2.3	2.7	2.2	2.9	33.7
Do.....	Fallow.....	15.6	14.5	10.7	5.5	7.2	5.9	59.4
Summer.....	Alfalfa.....	7.1	2.5	2.5	1.9	2.1	.4	16.5
Do.....	Oats.....	2.2	2.0	3.2	1.2	1.9	1.8	12.1
Do.....	Corn.....	1.2	2.9	8.5	4.1	4.0	1.4	22.1
Do.....	Potatoes.....	2.6	2.0	12.8	7.9	8.4	4.3	38.0
Do.....	Fallow.....	4.8	6.9	5.3	9.4	8.6	7.2	42.2
Fall.....	Alfalfa.....	8.5	4.2	2.4	3.5	2.8	2.0	23.4
Do.....	Oats.....	5.6	3.0	4.0	2.8	2.5	2.5	20.4
Do.....	Corn.....	3.0	3.6	2.7	2.0	2.7	2.4	16.4
Do.....	Potatoes.....	9.9	6.1	3.7	4.9	3.0	3.9	31.5
Do.....	Fallow.....	9.4	7.2	7.8	9.0	6.3	5.3	45.0

There is a marked difference in the quantity and distribution of the nitric nitrogen found in the soils growing the various crops. During the spring the nitric-nitrogen content of the surface feet of the alfalfa and corn land is comparatively low, while that of the oat, potato, and fallow soil is high. This is especially marked in the case of the last two, for here we find over one-half of the total existing within the 6 feet found within the first two. But in the summer the nitric nitrogen is carried to lower depths by the irrigation water, only to concentrate at the surface again during the fall. The total quantity of nitric nitrogen in the alfalfa, oat, and corn soil is low throughout the year, while the quantity in the potato and fallow is comparatively high. It is interesting to note that, in spite of the heavy drain which has been made upon the soil-growing crops, the nitric nitrogen in the soil during the fall is nearly the same as during the spring, while the fallow soil shows a loss of 14.4 pounds during summer. This is probably due to the water carrying the nitric nitrogen below 6 feet, and the results herein reported point strongly to the conclusion that the continuous application of 37.5 inches of irrigation water to a soil yearly is going to result in the loss of considerable nitrogen from that soil.

If we assume that all of the nitrogen which is removed by the oats or corn is nitrified before removal and then compare these results with those obtained on the fallow soil, we find that the fallow soil is short 77 pounds of nitric nitrogen. This quantity minus the extra quantity formed, due to the stimulating action of the plant upon the nitrifying organisms, may be taken as the minimum quantity which is leached out of this plot during the season or converted into other forms by bacteria. The loss from leaching in the case of the cropped plots would be much lower, but even here the loss from leaching where excessive quantities of water are used is considerable.

PLOTS RECEIVING 25 INCHES OF IRRIGATION WATER

There were five plots in this series, so arranged that the cropped plots can be compared with each other, and these in turn with the fallow. Since the treatment which the plots in this series have received, such as plowing, cultivation, etc., has been as nearly uniform as possible, the variable is the crop. The marked difference, therefore, in the several plots must be due to the influence of the crop upon the movement and production of nitric nitrogen. The average results for the three years are given in Table XVI.

TABLE XVI.—*Nitric nitrogen in soil with various crops and receiving 25 inches of irrigation water—Average for three years*

[Results expressed in pounds per acre]

Period.	Crop.	Depth of soil.						Total.
		1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
Spring.....	Alfalfa.....	1.9	2.0	1.8	1.4	2.5	1.4	11.0
Do.....	Oats.....	15.8	4.9	3.4	5.3	1.8	4.3	35.5
Do.....	Corn.....	7.2	2.3	2.0	1.9	1.9	2.2	17.5
Do.....	Potatoes.....	17.1	8.2	8.9	7.9	5.0	2.8	49.9
Do.....	Fallow.....	10.0	6.0	4.8	5.5	10.5	19.4	56.3
Summer.....	Alfalfa.....	2.4	3.2	2.3	1.8	1.4	1.5	12.6
Do.....	Oats.....	2.6	1.7	3.2	2.8	2.4	3.3	16.0
Do.....	Corn.....	1.7	1.6	5.5	3.9	1.9	2.2	16.8
Do.....	Potatoes.....	9.8	4.4	8.9	6.3	6.7	8.3	44.4
Do.....	Fallow.....	3.5	13.4	13.1	15.2	19.6	7.9	72.7
Fall.....	Alfalfa.....	14.9	9.0	3.2	2.4	2.0	4.4	35.9
Do.....	Oats.....	4.0	3.2	3.0	2.7	2.8	2.6	18.3
Do.....	Corn.....	3.7	3.0	2.2	2.7	2.4	3.2	17.2
Do.....	Potatoes.....	18.9	5.5	6.2	7.9	5.2	4.2	47.9
Do.....	Fallow.....	16.5	7.9	9.7	6.0	7.8	4.5	52.4

It may be seen that during the spring the nitric nitrogen of the alfalfa soil is very low, and the quantity present is about equally distributed throughout the 6 feet, while in the other plots the total quantity is much higher. Especially is this true in the potato and fallow soil, and we

find the greater part of it concentrated in the surface-foot sections. During the summer it is more evenly distributed throughout the 6 feet. The fallow gains considerable nitric nitrogen during the early summer, while each of the other soils shows a loss, which in the case of the oat soil is quite pronounced. Prior to the summer sampling it appears that the water applied has not been sufficient to carry much of the nitric nitrogen below the sixth foot, but during the time which elapsed between the summer and fall sampling there was a loss from the fallow soil, with a slight gain in each of the other soils. Moreover, these results, taken in connection with those previously published by us, where samples were taken to a depth of 10 feet, indicate that no small quantity of the nitric nitrogen is carried below the sixth-foot level. Hence, the continuous use of this quantity of water on these plots would result in an unnecessary loss of nitric nitrogen from the soil. The fallow, potato, and corn land contains practically the same quantity of nitric nitrogen in the soil during the spring as during the fall, while the alfalfa and oats show a wide variation.

#### PLOTS RECEIVING 15 INCHES OF IRRIGATION WATER

Each of the plots in this series received 15 inches of irrigation water. The treatment of each plot was as nearly similar as compatible with the various crops growing upon them; hence, any difference in nitric nitrogen noted should be due to the crops. The average summarized results for the three years are given in Table XVII.

TABLE XVII.—*Nitric nitrogen in soil with various crops and receiving 15 inches of irrigation water—Average for three years*

[Results expressed as pounds per acre]

Period.	Crop.	Depth of soil.						Total.
		1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
Spring.....	Alfalfa.....	3.4	0.9	11.6	3.3	1.6	1.9	22.7
Do.....	Oats.....	7.6	6.9	9.6	4.6	1.7	3.0	33.4
Do.....	Corn.....	12.3	8.5	4.1	2.0	1.6	1.9	30.4
Do.....	Potatoes.....	20.5	8.7	4.0	4.0	3.1	4.0	44.3
Do.....	Fallow.....	10.4	15.6	9.2	9.1	13.6	9.5	67.4
Summer.....	Alfalfa.....	5.4	.8	2.1	1.7	3.1	1.4	15.1
Do.....	Oats.....	5.1	3.8	3.7	1.7	3.0	.8	18.1
Do.....	Corn.....	1.4	5.2	3.7	2.5	3.2	3.3	19.3
Do.....	Potatoes.....	16.1	15.2	7.2	3.2	3.0	2.4	47.1
Do.....	Fallow.....	8.4	6.2	4.4	2.6	2.1	3.1	26.8
Fall.....	Alfalfa.....	10.7	10.4	4.4	2.3	2.9	1.8	32.5
Do.....	Oats.....	4.2	3.3	4.1	3.4	2.9	2.7	20.6
Do.....	Corn.....	5.8	2.5	3.2	3.0	3.2	3.2	20.9
Do.....	Potatoes.....	10.9	3.7	2.5	6.6	7.2	4.4	35.3
Do.....	Fallow.....	10.1	7.4	6.3	7.2	7.7	7.3	46.0



The quantity of nitric nitrogen found in the alfalfa and oat soil is low during the spring and it is quite evenly distributed throughout the 6 feet, while the corn, potato, and fallow soil is exceptionally high in nitric nitrogen in the first foot. The total quantity of nitric nitrogen in the potato and fallow soil throughout the season is higher than in the other plots, but in the case of the fallow there is a marked decrease in the total nitric nitrogen of the soil. This must be due to the action of bacteria in transforming the nitrates into protein material within the soil, because it is not likely that this quantity of water would be sufficient to carry the nitric nitrogen beyond the 6 feet. Furthermore, if we compare the nitrates found in the soil for the last three years with those for the previous eight years, we find that during the last three years the total quantity of nitric nitrogen in the 6 feet of soil is one-third lower than it was during the first period, showing a decrease in the nitric nitrogen of fallow soil. Whether this can be due to a decrease in the total nitrogen of the soil or merely to a decrease in the nitrifying powers of the soil can not be answered with the data at hand. There is the possibility that in the presence of large quantities of nitrates there may be developed a strain of bacteria which would rapidly transform the ammonia and nitrates into protein material. It does not seem possible that any great quantity of the nitrogen could have disappeared through denitrification, for the soil is well aerated and the quantity of organic matter present is extremely low.

#### LOTS RECEIVING NO IRRIGATION WATER

All of the plots in this series were treated as nearly uniform as possible. The oats and alfalfa plots were not cultivated. The plots were unirrigated, and the marked difference in the nitric nitrogen of the various plots is probably due to the crop factor. The average summarized results for the three years are given in Table XVIII.

The nitric nitrogen of all the unirrigated plots is comparatively high in the spring, decreases in the summer, and increases in the fall. The greatest decrease occurs in those plots having the greatest accumulation of nitrates. In the potato plots the unaccounted-for nitrates amount to 82.6 pounds per acre, only slightly greater than in the fallow, which shows a loss of 71.2 pounds. The fact that this loss is so pronounced in the fallow soil shows that it is not due to the removal of the nitric nitrogen by the growing plants. The fact that it is so rapidly regained during the winter months clearly indicates that it is not due to water removing the soluble nitrates; nor is it due to denitrification, for these plots are as high in total nitrogen as are others which have not shown this seasonal loss of nitrates. The probable explanation of the phenomena which we have so continuously observed in these plots is the following. The accumulation of large quantities of nitrates in the soil depresses to a degree the speed with which the nitrifying organisms act, but it increases

the speed with which the protein-forming organisms work. Further more, while the increased temperature of summer increases the activity of both classes of organisms, the speed of the latter is accelerated to a much greater extent by heat than is the speed of the former. Moreover, both classes of organisms are sensitive to cold, but it would appear that those which bring about synthetic reactions are much more sensitive than are those which bring about the analytic change. We therefore have an accumulation of nitrates during the cold seasons and a disappearance during the warmer months of the year. These facts would be in keeping with the findings of Conn, Brown, and others who have noticed that cold increases the nitrifying powers of the soil.

TABLE XVIII.—*Nitric nitrogen in soil with various crops and receiving no irrigation water—Average for three years*  
[Results expressed as pounds per acre]

Period.	Crop.	Depth of soil.						Total.
		1st foot.	2d foot.	3d foot.	4th foot.	5th foot.	6th foot.	
Spring.....	Alfalfa.....	10.9	5.1	11.4	2.2	9.3	6.0	44.9
Do.....	Oats.....	9.1	5.9	4.0	6.1	10.7	6.3	42.1
Do.....	Corn.....	12.0	4.1	1.9	2.5	3.4	3.5	27.4
Do.....	Potatoes.....	10.9	29.0	43.6	46.4	38.5	27.9	196.3
Do.....	Fallow.....	18.9	39.6	31.9	14.8	21.7	16.1	143.0
Summer.....	Alfalfa.....	3.3	8.8	2.2	1.4	2.6	1.0	19.3
Do.....	Oats.....	3.3	1.6	1.5	1.6	1.6	.8	10.4
Do.....	Corn.....	3.3	3.1	2.9	2.6	3.2	2.2	17.3
Do.....	Potatoes.....	25.9	8.9	17.5	25.1	18.4	17.9	113.7
Do.....	Fallow.....	15.5	9.7	8.2	9.7	13.6	16.1	72.8
Fall.....	Alfalfa.....	19.5	7.0	6.5	3.0	2.1	1.1	39.2
Do.....	Oats.....	6.7	3.9	3.7	3.3	2.9	2.8	23.3
Do.....	Corn.....	11.2	4.6	3.1	3.5	5.0	5.9	33.3
Do.....	Potatoes.....	34.6	17.6	9.8	11.5	19.1	9.6	102.2
Do.....	Fallow.....	31.5	17.6	18.1	15.9	11.5	12.3	106.9

If we average the nitric nitrogen in the plots growing the various crops, we find a marked difference in the quantity found in the various plots (Table XIX).

TABLE XIX.—*Average nitric nitrogen in the plots growing various crops*  
[Results expressed as pounds per acre]

Crop.	Time of sampling.			Average.
	Spring.	Summer.	Fall.	
Alfalfa.....	22.3	15.8	32.8	23.6
Oats.....	35.7	14.1	20.6	23.5
Corn.....	24.8	18.9	22.0	21.9
Potatoes.....	81.1	60.8	54.2	65.3
Fallow.....	81.5	53.6	62.6	65.9

Here we find a much smaller quantity of nitric nitrogen in all the plots during the summer than during either spring or fall; and with one exception, the alfalfa, it is lower in the fall than in the spring. During the summer months we find the alfalfa and oats much closer feeders on the nitric nitrogen of the soil than are the other plants. However, the average quantity of nitric nitrogen found in the 6 feet of soil for the season on the alfalfa, oat, and corn soil is nearly the same in each case. This appears to contradict the conclusions previously reached by us; but it must be borne in mind that in the previous work our samples were taken to a depth of 10 feet, and a comparison of the two sets of results indicates that the alfalfa and oats, besides being closer feeders upon nitric nitrogen, feed to a greater depth than do the other crops. Furthermore, it makes it very certain that in a study of the nitric nitrogen of a soil, such as used in these experiments, samples must be taken to a great depth; otherwise erroneous conclusions will be drawn from the results obtained.

It is interesting to note that throughout the season there is over twice the nitric nitrogen in the potato and fallow soil than in any of the other soils, and even these plots show a decrease in nitric nitrogen during summer and fall. There is a slight difference in favor of the fallow plots in the fall, but in the spring the quantity in both sets is the same.

#### COMPOSITION OF THE SOIL SOLUTION

Moisture determinations were made on each soil at time of sampling so the results could be calculated to a dry basis. From the results obtained for nitric nitrogen and soil moisture, it is possible to calculate the concentration of the soil solution. While this has been done for each individual plot, only the summarized results are reported in Tables XX and XXI, and they represent the average for all the determinations covering these years.

TABLE XX.—*Concentration of the soil solution growing various crops*

[Results reported as nitric nitrogen parts per million of soil solution]

Crop.	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.	Average.
Alfalfa.....	16.0	10.8	9.8	5.2	6.7	4.7	8.87
Oats.....	14.0	7.6	8.7	6.8	7.0	6.4	8.92
Corn.....	13.3	7.7	7.5	6.2	6.9	6.6	8.03
Potatoes.....	32.6	21.6	22.3	24.0	24.1	15.2	23.28
Fallow.....	26.4	25.3	23.5	18.7	20.6	19.7	22.38

It is interesting to note the great difference between the concentration of the soil solution of the alfalfa and potato soils. The average concentration of the alfalfa, oat, and corn land is about the same, while the potato and fallow is the same. However, there is a slight difference in

the concentration of the surface foot in favor of the potato soil. But these results show conclusively that the soil solution is influenced to a depth of at least 6 feet by the crop grown upon it.

TABLE XXI.—*Concentration of the soil solution where various quantities of water have been applied*

[Results reported as nitric nitrogen parts per million of soil solution]

Water applied (inches).	First foot.	Second foot.	Third foot.	Fourth foot.	Fifth foot.	Sixth foot.	Average.
37.5.....	14.2	9.9	10.0	8.2	8.1	6.7	9.5
25.....	19.7	10.2	10.4	10.4	10.5	11.6	12.1
15.....	16.8	13.4	11.2	8.1	8.6	7.2	10.9
None.....	35.6	27.5	28.0	25.2	30.0	24.8	28.5

The concentration of the soil solution varies with the quantity of water applied to the soil during the season. It is three times as concentrated in the soil which received no irrigation water as in the soil which received 37.5 inches; but the difference is not great between the concentration of the heaviest irrigated soil and those which received much smaller quantities of water.

#### INFLUENCE OF WATER ON THE NUMBER OF ORGANISMS AND ON THE AMMONIFYING AND NITRIFYING POWERS OF THE SOIL

All of the plots which have been considered in the previous discussion were sampled, and bacteriological analyses made of the soils. The samples for this work were taken on the same day in the spring, midsummer, and fall as were the samples for the direct chemical analyses. These, however, were taken to a depth of only 12 inches. Three individual determinations were made at each sampling during each season for the three years, so each result as reported, unless stated to the contrary, represents the average of nine or more analyses. Determinations were made of the number of organisms developing upon synthetic agar, the ammonifying powers, and the nitrifying powers of the soil. The results have been so arranged that we can compare the soil from each of the plots receiving the various quantities of water with each other and these in turn with the unirrigated. Furthermore, it is possible to compare directly the number of organisms, ammonia, and nitric nitrogen produced, as all are reported in the same table (Table XXII).

#### ALFALFA

There were four plots in the alfalfa series. These received varying quantities of water; otherwise, they were all treated alike. To one plot were applied 37.5 inches of water; to another, 25 inches; to a third, 15 inches, while one was unirrigated. The average results for the three years are given in Table XXII.

TABLE XXII.—Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil receiving varying amounts of water. Crop, alfalfa. Average for three years

NUMBER OF COLONIES OF BACTERIA DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR PER GRAM OF SOIL

Plot No.	Water applied.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
	<i>Inches.</i>				
31.....	37.5	6,700,000	6,433,000	2,800,000	5,311,000
32.....	25.0	8,933,000	7,066,000	4,933,000	6,977,000
33.....	15.0	9,466,000	6,566,000	4,600,000	6,877,000
34.....	None.	8,133,000	6,333,000	5,667,000	6,711,000

MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

31.....	37.5	50.5	46.0	42.2	46.2
32.....	25.0	49.9	51.2	39.0	46.7
33.....	15.0	51.9	52.8	35.2	46.6
34.....	None.	53.5	52.7	43.0	49.7

MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

31.....	37.5	3.7	9.9	1.9	5.2
32.....	25.0	2.7	8.6	3.7	5.0
33.....	15.0	4.7	5.2	3.6	4.5
34.....	None.	1.5	6.2	3.1	3.6

The number of organisms in this soil which develop on synthetic agar is greater in May than in August or November. In all the plots there was a gradual decrease from spring to fall. This difference is greatest in those plots which received the most water. The number of organisms is greatest in the soil which received no water and least in the soil which received 37.5 inches of water. In every instance during spring and fall the number of organisms decreases as the water applied increases; and the difference is so marked and regular that it seems safe to attribute it to the water applied. During the summer the difference in the number of organisms in the various plots is not great; especially is this true in the irrigated plots.

The ammonifying powers of all the soils are highest in spring and lowest in fall. The difference in the quantity of ammonia produced in the various soils is not great. But during the spring and summer the ammonifying powers of each soil decrease as the water applied increases. The difference is not regular in the fall; but from all the results it seems quite certain that the addition of irrigation water to alfalfa soil, such as used in this investigation, causes a decrease in the ammonifying powers of the same.

The nitrifying powers of all the soils are higher in midsummer and lower in the fall and spring. This difference is very pronounced in the soil



receiving the greatest quantities of water. During the spring and summer the nitrifying powers of the soil are quite regularly increased by the irrigation water. But apparently the water in the soil where the 37.5 inches are being applied toward fall becomes sufficient to depress greatly its ammonifying powers. This, however, may be due to the continual washing of the nitrifying organisms to below 12 inches.

There is a marked relationship between the number of organisms and the ammonifying powers of the soil, but the nitrifying powers show no relationship to either.

#### POTATO LAND

The plots in this series were each planted to potatoes, and all received the same cultivation and general treatment, with the exception of water applied, which varied from no irrigation to 37.5 inches per year. The average results for the three years are given in Table XXIII.

TABLE XXIII.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil receiving varying amounts of water. Crop, potatoes. Average for three years*

#### NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR PER GRAM OF SOIL

Plot No.	Water applied.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
	<i>Inches.</i>				
35.....	37.5	4,933,000	5,766,000	4,533,000	5,077,000
36.....	25.0	5,666,000	7,000,000	4,000,000	5,555,000
37.....	15.0	5,833,000	5,067,000	4,800,000	5,233,000
38.....	None.	7,167,000	6,500,000	5,833,000	6,500,000

#### MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

35.....	37.5	50.3	67.9	53.3	57.2
36.....	25.0	63.0	66.3	48.5	59.2
37.....	15.0	51.2	57.4	38.7	49.1
38.....	None.	56.4	65.0	50.6	57.3

#### MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

35.....	37.5	4.0	10.2	2.2	5.5
36.....	25.0	4.3	17.8	10.4	10.8
37.....	15.0	2.6	20.6	6.0	9.7
38.....	None.	1.1	13.6	3.8	6.2

The relationship between the water applied and the number of bacteria is not as well defined in the potato as in the alfalfa soil; but even in the potato plots the tendency is for the larger quantities of water to depress the number of bacteria. The number of organisms is slightly higher in the spring than in the fall, and the difference which is noted in the fall appears again in the spring. The number found in the summer is considerably higher than the number found in either fall or spring. The results as a whole indicate that the water has decreased the number of organisms in the first foot.

During the spring the ammonifying power of the soil which had received 25 inches of water is considerably higher than any of the others, while in the fall and summer the soil which received 37.5 inches of water is the greatest. The results collectively indicate that the irrigation water has increased the ammonifying powers of the potato soil. The ammonifying powers of all the plots are higher in summer than in either spring or fall.

The nitrifying powers of the soil are much higher in summer than either fall or spring, and there is a relationship between the water applied and the nitrifying powers of the soil. The water increases the nitrifying powers of the soil. The highest results were obtained where 25 inches of water were applied.

For the potato land we note a decrease in the number of organisms as the water applied increases, but an increase in both ammonifying and nitrifying powers with increased water. So it would appear that the water has increased the physiological efficiency of the organisms without increasing the number.

#### OAT LAND

The oat plots received 37.5 inches, 25 inches, 15 inches, and no water. Otherwise they were all treated the same. The average results for the three years are given in Table XXIV.

TABLE XXIV.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil receiving varying amounts of water. Crop, oats. Average for 3 years*

NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR PER GRAM OF SOIL

Plot No.	Water applied.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
	<i>Inches.</i>				
43.....	37.5	6, 133, 000	6, 000, 000	4, 133, 000	5, 422, 000
44.....	25.0	5, 700, 000	4, 800, 000	2, 866, 000	4, 455, 000
45.....	15.0	6, 200, 000	5, 133, 000	4, 400, 000	5, 244, 000
46.....	None.	7, 533, 000	5, 967, 000	6, 000, 000	6, 500, 000

MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

43.....	37.5	56.8	69.3	49.6	58.6
44.....	25.0	53.3	56.3	52.1	53.9
45.....	15.0	48.0	55.4	46.0	49.8
46.....	None.	48.8	50.2	44.7	47.9

MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

43.....	37.5	1.7	3.9	2.1	2.6
44.....	25.0	2.2	4.7	.8	2.6
45.....	15.0	1.7	2.0	2.3	2.0
46.....	None.	4.0	5.4	6.8	5.4

In every case the number of organisms is greater in the oat soil during the spring than in summer or fall, and with one exception they are greater in summer than in fall. While the difference is not as great nor as regular as might be desired, the tendency seems to be for the water to depress the number of organisms in oat soil.

The ammonifying powers of the soil is higher in the summer than in the fall or spring, and there is a pronounced and regular difference between those receiving the various quantities of irrigation water in favor of those receiving the greatest quantity. While the difference is considerable throughout the season, it is more pronounced in the summer than at the time of taking the other samples. From an examination of the nitrification series one sees that this apparent increase in ammonia, due to water, may be caused in part by the depression of the nitrifying organisms, for the application of irrigation water to these soils has depressed the nitrifying powers of the soil. This is quite pronounced at each season, and appears in the results for each specific year. Hence, it must be attributed to the water applied.

## CORN LAND

The four corn plots in this series received 37.5 inches, 25 inches, 15 inches, and no irrigation water. Otherwise, they were all handled the same. The average summarized results for the three years are given in Table XXV.

TABLE XXV.—Number of colonies of bacteria, milligrams of ammonia, and milligram of nitric nitrogen from soil receiving varying amounts of water. Crop, corn. Average for 3 years

## NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR PER GRAM OF SOIL

Plot No.	Water applied.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
	<i>Inches.</i>				
47.....	37.5	6,700,000	6,866,000	3,333,000	5,633,000
48.....	25.0	4,600,000	5,800,000	3,366,000	4,589,000
49.....	15.0	5,933,000	6,133,000	2,967,000	5,011,000
50.....	None.	6,100,000	3,867,000	4,767,000	4,911,000

## MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

47.....	37.5	50.5	59.1	46.7	52.1
48.....	25.0	55.2	55.8	44.4	51.8
49.....	15.0	50.5	61.5	43.2	51.7
50.....	None.	55.2	57.2	46.6	53.0

## MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

47.....	37.5	2.6	7.6	1.5	3.9
48.....	25.0	1.9	2.1	0.5	1.5
49.....	15.0	2.3	2.2	2.4	2.3
50.....	None.	1.9	2.1	1.5	1.8

The number of organisms in the corn soil is highest in summer and lowest in the fall. But during spring and fall there would appear to be no relationship between the water applied and number of organisms. However, in the summer there is a very pronounced difference in favor of the plots receiving the greatest quantity of water, and this regularity is found in each season's results.

The results obtained for ammonification are very irregular, but it is interesting to note that they in every case have almost a quantitative relationship to the results obtained with the potatoes.

Nitrification is slightly higher in the summer than during either of the other seasons, and it would appear that the irrigation water had increased the nitrifying powers.

#### FALLOW SOIL

The fallow plots received the same quantity of water as the cropped plots and were plowed and handled the same. Any weeds which appeared during the summer were pulled. The average summarized results for the three years are given in Table XXVI.

TABLE XXVI.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil receiving varying amounts of water. Crop, fallow. Average for three years*

#### NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR PER GRAM OF SOIL

Plot No.	Water applied.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
	<i>Inches.</i>				
39.....	37.5	8,266,000	6,933,000	2,200,000	5,800,000
40.....	25.0	3,300,000	3,370,000	3,700,000	3,457,000
41.....	15.0	4,867,000	3,967,000	2,533,000	3,789,000
42.....	None.	3,100,000	3,633,000	1,767,000	2,833,000

#### MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

39.....	37.5	66.2	69.4	49.6	61.7
40.....	25.0	66.8	80.1	56.1	67.7
41.....	15.0	61.5	76.8	46.5	61.6
42.....	None.	61.2	56.7	52.6	56.8

#### MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS.

39.....	37.5	1.5	8.7	1.1	3.7
40.....	25.0	.6	2.0	1.5	1.3
41.....	15.0	1.5	3.6	3.1	2.7
42.....	None.	1.6	7.7	4.2	4.5

The number of organisms in these plots is highest in spring and lowest in fall, and they show an increase due to the irrigation water used. The ammonifying powers are highest in summer and lowest in fall, and the water applied increases the ammonifying power and is greatest where 25 inches are applied. The 37.5 inches, while they are better than none, depress the ammonifying powers when compared with 25 inches.

It was thought likely that this decrease in the nitrifying powers and formation of nitric nitrogen in these plots with highest water content may be due to a difference in temperature. For this reason temperature determinations were made on the soil during September and October of one season. The determinations were made to a depth of 4 feet with soil thermometers placed in the ground and read twice daily, at 8 a. m. and 5 p. m. The average results for the season are given in Table XXVII, expressed in degrees Fahrenheit.

TABLE XXVII.—Average temperature (°F.) of the soil at different depths

Plot.	Time.	First foot.	Second foot.	Third foot.	Fourth foot.
39	8 a. m. ....	54.8	52.1	49.9	47.2
	5 p. m. ....	55.1	52.3	49.8	48.7
40	8 a. m. ....	53.9	52.6	47.4	.....
	5 p. m. ....	53.9	52.7	48.9	.....
41	8 a. m. ....	54.3	53.3	51.8	49.1
	5 p. m. ....	54.7	53.1	51.7	49.4
43	8 a. m. ....	56.5	55.4	52.8	50.3
	5 p. m. ....	56.8	55.6	52.6	51.2

This table shows that the temperature is about 2 degrees higher in the nonirrigated soil than in the soil receiving the greatest quantity of water; and the difference is about the same even in the fourth foot, which has a temperature about 6 degrees lower than the temperature of the surface soil. The temperature of the soil is only slightly different in morning and evening, but the difference extends to a depth of 4 feet.

The nitrifying powers are highest in midsummer, at which time they are apparently increased by the irrigation water, but are depressed by the larger quantities in the fall.

The results which we have considered indicate that the irrigation water applied has clearly depressed the number of organisms which develop upon synthetic agar in alfalfa, oats, and potato soil. The results obtained for the corn are not regular, but there is a marked increase in the fallow.

The ammonifying powers were increased in all of the soils except the alfalfa, and in this case there was a decrease.

The nitrifying powers have been increased in every case except in the oat soil. The average results for the different water treatments are given in Table XXVIII.



TABLE XXVIII.—Average bacterial activities in soil with various water treatments

## NUMBER OF COLONIES OF BACTERIA

Water applied (inches).	Spring.	Summer.	Fall.	Average.
37.5.....	6, 546, 000	7, 600, 000	3, 400, 000	5, 849, 000
25.0.....	5, 640, 000	5, 606, 000	3, 773, 000	5, 006, 000
15.0.....	6, 260, 000	5, 173, 000	3, 860, 000	5, 098, 000
None.....	6, 206, 600	6, 060, 000	4, 807, 000	5, 691, 000

## MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL

37.5.....	54.8	62.3	46.3	54.5
25.0.....	57.2	61.9	48.0	55.7
15.0.....	52.6	60.8	40.9	51.4
None.....	55.0	58.2	47.5	53.6

## MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL

37.5.....	2.7	8.4	1.7	4.3
25.0.....	2.3	7.0	3.4	4.2
15.0.....	2.5	6.7	3.8	4.3
None.....	2.0	7.0	3.9	4.3

If we take all the results into consideration, it is clear that the irrigation water had increased all of the bacterial activities of the soil; but it will be noted that the numbers of organisms, the ammonifying powers, and nitrifying powers of all the plots are extremely low; and it would appear that in all these plots the limiting factor is the organic matter. Had there been more organic matter present, the effect of the water would have been more pronounced, as it was found by one of us (21) in other experiments to be the case.

Furthermore, the difference which actually exists in the bacterial activities must have been greater than is brought out by these results, for during one season we made determinations of the number of organisms in the soil, and the ammonifying powers and the nitrifying powers of the soil both the day before irrigation and the day after irrigation. The results for the day before irrigation averaged one-fourth higher than did the results for the day after irrigation, thus clearly indicating that many of the organisms and the different species in about the same proportion had been carried below the first foot by the irrigation waters. This being the case, in order that an increase for the bacterial activities for the season is obtained, the remaining organisms must have multiplied much faster, or their physiological efficiency have become much greater in the irrigated than in the unirrigated. There is the possibility that the organisms would rapidly be brought to the sur-

face again by the water, but we could not expect this to be as great for the bacteria as it is for the soluble nitrates, and it has been seen that, even in the case of these where the greater quantities of water were used, the nitrates never concentrate in the surface foot during the summer, the results showing a decrease in most plots, due to water. It is therefore quite reasonable to expect that bacteria which developed upon synthetic agar would be carried down in about the same proportions as were the other organisms; hence, the increased bacterial activities noted must be due to an increased physiological efficiency of the organisms. Moreover, had samples been taken to a depth of 3 feet for the bacteriological analysis, we would have obtained just as pronounced an effect upon number of colonies of bacteria, ammonifying, and nitrifying powers of the soil as we have in the case of the nitrates, which is the summation effect noted in the 6 feet.

During the first season determinations were made of the nitrogen-fixing powers of the soil, but during the succeeding years we were so crowded with other work that it became impossible to continue this phase of the work. While the results for one season are not sufficient to warrant their consideration in detail, the average results are of interest, as they show the best fixation where 15 inches of water were applied to the soil. The averages for the various treatments were as follows:

37.5 inches of water.....	1.4 mgm. of nitrogen fixed in 100 gm. of soil.
25 inches of water.....	2.1 mgm. of nitrogen fixed in 100 gm. of soil.
15 inches of water.....	8.5 mgm. of nitrogen fixed in 100 gm. of soil.
None.....	3.5 mgm. of nitrogen fixed in 100 gm. of soil.

#### INFLUENCE OF CROP WITH THE DIFFERENT QUANTITIES OF WATER ON BACTERIAL ACTIVITIES

The experiment was so planned that, besides giving information upon the influence of water upon the bacterial activities of the soil, it should also give definite information upon the influence of crop on these same properties. This being the case, the results have been rearranged so that the crop is the variable and the quantity of water a constant. We can therefore compare the results from the alfalfa with the various quantities of water with those obtained where other crops receiving like amounts of water were grown, and each of these in turn can be compared with the fallow.

##### PLOTS RECEIVING 37.5 INCHES OF IRRIGATION WATER

In this series the alfalfa, oats, corn, potato, and fallow soil each received 37.5 inches of irrigation water. The average summarized results for the three years are given in Table XXIX.

The number of organisms in the soil is greatest in the spring and least in the fall. During the spring the fallow has many more organisms than any of the cropped soils. During the summer the cropped and uncropped plots contain about the same number of organisms, while in the fall all

of the cropped plots contain a greater number of organisms than does the fallow, which would indicate that the limiting factor is more pronounced in its effect in fall on fallow soil than on cultivated soil.

TABLE XXIX.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil with various crops receiving 37.5 inches of irrigation water*

NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR

Plot No.	Crop.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
31.....	Alfalfa.....	6, 700, 000	6, 433, 000	2, 800, 000	5, 311, 000
43.....	Oats.....	6, 133, 000	6, 000, 000	4, 133, 000	5, 422, 000
47.....	Corn.....	6, 700, 000	6, 866, 000	3, 333, 000	5, 633, 000
35.....	Potatoes....	4, 933, 000	5, 766, 000	4, 533, 000	5, 077, 000
39.....	Fallow.....	8, 266, 000	6, 933, 000	2, 200, 000	5, 799, 000

MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

31.....	Alfalfa.....	50. 5	46. 0	43. 2	46. 6
43.....	Oats.....	56. 8	69. 3	49. 6	58. 6
47.....	Corn.....	50. 5	59. 1	46. 7	52. 1
35.....	Potatoes....	50. 3	67. 9	53. 3	57. 1
39.....	Fallow.....	66. 2	69. 4	49. 6	61. 7

MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

31.....	Alfalfa.....	3. 7	9. 9	1. 9	5. 2
43.....	Oats.....	1. 7	3. 9	2. 1	2. 6
47.....	Corn.....	2. 6	7. 6	1. 5	3. 9
35.....	Potatoes....	4. 0	10. 2	2. 2	5. 5
39.....	Fallow.....	1. 5	8. 7	1. 1	3. 7

The ammonifying powers of the soils are higher in summer than either spring or fall, and they are much higher in the fallow than in any of the cropped soils, the alfalfa being the lowest. In the fall the difference is not as marked, but even here the potato soil is the only one which has higher ammonifying powers than has the fallow. We thus find a close relationship between numbers and ammonifying powers of the soil. With the nitrifying powers we find no such regularity. Here the highest results are obtained with the potatoes, with the alfalfa a close second. Moreover, these results, if they represent what actually occurs in field conditions, indicate that the alfalfa must be removing the nitrogen from the soil much more rapidly than any of the other crops, for our previous results have taught us that the alfalfa is a close feeder upon the soluble nitric nitrogen of the soil, and now we find alfalfa soil nitrifying the organic material to a greater extent than do the other soils.

The low nitrifying powers of the oat soil is significant, as it indicates that the small quantities of nitric nitrogen found in this soil may be due in part to this factor.

## PLOTS RECEIVING 25 INCHES OF IRRIGATION WATER

The four cropped plots and one fallow plot were in this series, and each received 25 inches of irrigation water during the season. The summarized results are given in Table XXX.

TABLE XXX.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil with various crops receiving a medium application of water (25 inches)*

## NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR

Plot No.	Crop.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
32.....	Alfalfa.....	8, 933, 000	7, 066, 000	4, 933, 000	6, 977, 000
44.....	Oats.....	5, 700, 000	4, 866, 000	2, 866, 000	4, 477, 000
48.....	Corn.....	4, 600, 000	5, 800, 000	3, 366, 000	4, 589, 000
36.....	Potatoes.....	5, 666, 000	7, 000, 000	4, 000, 000	5, 555, 000
40.....	Fallow.....	3, 300, 000	3, 370, 000	3, 700, 000	3, 457, 000

## MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

32.....	Alfalfa.....	47. 9	51. 2	39. 0	46. 0
44.....	Oats.....	53. 3	56. 3	52. 1	53. 9
48.....	Corn.....	55. 2	55. 8	44. 4	51. 8
36.....	Potatoes.....	63. 0	66. 3	48. 5	59. 2
40.....	Fallow.....	66. 8	80. 1	56. 1	67. 6

## MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

32.....	Alfalfa.....	2. 7	8. 6	3. 7	5. 0
44.....	Oats.....	2. 2	4. 7	. 8	2. 6
48.....	Corn.....	1. 9	2. 1	. 5	1. 5
36.....	Potatoes.....	4. 3	17. 8	10. 4	10. 8
40.....	Fallow.....	. 6	2. 0	1. 5	1. 3

The number of organisms in the alfalfa soil is very high during the spring and very low and uniform in the fallow throughout the year. The greatest difference in the plots is noted in the spring, the numbers being more nearly uniform during the summer; especially is this the case in the fall.

A very marked difference is noted in the ammonifying powers of the soils in favor of the fallow. Naming the plots in the order of increasing efficiency, we have alfalfa, oats, corn, potatoes, and fallow, which is about the order they have been maintaining in each series. And the difference is very pronounced in favor of the fallow soil. But we find the nitrifying powers of the fallow soil very low. In this series during the season it is high in the potato soil. The alfalfa is high compared with any of the other soils, thus showing that alfalfa soil has a high nitrifying power when compared with oats, corn, or even fallow.

## PLOTS RECEIVING 15 INCHES OF IRRIGATION WATER

The treatments of these plots were, with the exception of the water applied (15 inches), the same as the previous series. The average results for the three years are given in Table XXXI.

TABLE XXXI.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil with various crops receiving a minimum application of water (15 inches)*

## NUMBER OF COLONIES DEVELOPED IN 4 DAYS ON SYNTHETIC AGAR

Plot No.	Crop.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
33.....	Alfalfa.....	9, 466, 000	6, 566, 000	4, 600, 000	6, 877, 000
45.....	Oats.....	6, 200, 000	5, 133, 000	4, 400, 000	5, 244, 000
49.....	Corn.....	5, 933, 000	6, 133, 000	2, 967, 000	5, 011, 000
37.....	Potatoes...	5, 833, 000	5, 067, 000	4, 800, 000	5, 233, 000
41.....	Fallow....	4, 867, 000	3, 967, 000	2, 533, 000	3, 789, 000

## MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

33.....	Alfalfa.....	51.9	52.8	35.2	46.6
45.....	Oats.....	48.0	55.4	46.0	49.8
49.....	Corn.....	50.5	61.5	43.2	51.7
37.....	Potatoes...	51.2	57.4	38.7	49.1
41.....	Fallow....	61.5	76.8	46.5	61.6

## MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

33.....	Alfalfa.....	4.7	5.2	3.6	4.5
45.....	Oats.....	1.7	2.0	2.3	2.0
49.....	Corn.....	2.3	2.2	2.4	2.3
37.....	Potatoes...	2.6	20.6	6.0	9.7
41.....	Fallow....	1.5	3.6	3.1	2.7

Here, again, we find the greater number of organisms in the soil during the spring, with a great decrease during the fall. But the greatest number of organisms are found in the alfalfa and the least in the fallow.

The ammonifying powers of all the soils are highest in summer and lowest in fall. The fallow soil has a higher ammonifying efficiency than any of the others, which is the same as the order noted where the maximum quantity of water was applied to the soil.

The nitrifying powers of the fallow soil are very low, and of the alfalfa soil high, thus bearing out the observation made for the previous series. The quantity of nitrates produced by the potato soil during the summer and fall is very high, and is probably due in a degree to the cultivation received by these plots. It appears in all of the potato plots and not in the corn plots, which were also cultivated; hence, it must be due in a measure to the crop.



## UNIRRIGATED SERIES

All of the crops which have appeared in the previous series, together with fallow, appear in the unirrigated series. The summarized results for the three years are given in Table XXXII.

TABLE XXXII.—*Number of colonies of bacteria, milligrams of ammonia, and milligrams of nitric nitrogen from soil with various crops receiving no irrigation water*

## NUMBER OF COLONIES DEVELOPING IN 4 DAYS ON SYNTHETIC AGAR

Plot No.	Crop.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
34.....	Alfalfa.....	8, 133, 000	6, 333, 000	5, 667, 000	6, 711, 000
46.....	Oats.....	7, 533, 000	5, 967, 000	6, 000, 000	6, 500, 000
50.....	Corn.....	6, 100, 000	3, 867, 000	4, 676, 000	4, 911, 000
38.....	Potatoes....	7, 167, 000	6, 500, 000	5, 833, 000	6, 500, 000
42.....	Fallow.....	3, 100, 000	3, 633, 000	1, 767, 000	2, 833, 000

## MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

34.....	Alfalfa.....	53.5	52.7	43.0	49.7
46.....	Oats.....	48.8	50.2	44.7	47.9
50.....	Corn.....	55.2	57.2	46.6	53.0
38.....	Potatoes....	56.4	65.0	50.6	57.3
42.....	Fallow.....	61.2	65.7	52.6	59.8

## MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

34.....	Alfalfa.....	1.5	6.2	3.1	3.6
46.....	Oats.....	4.0	5.4	6.8	5.4
50.....	Corn.....	1.9	2.1	1.5	1.8
38.....	Potatoes....	1.1	13.6	3.8	6.2
42.....	Fallow.....	1.6	7.7	4.2	4.5

The organisms are highest in the alfalfa and lowest in the fallow soil throughout the year. With the exception of spring and summer for the series which received the maximum quantity of water, the alfalfa soil has been much higher in bacteria than any of the other soils. And in most of the series the decrease in number from spring to fall is much more pronounced in the fallow than in any of the cropped soils, thus indicating that something develops in the fallow soil during the summer which limits the number of organisms. However, it is not the water applied, for we find the decrease just as great in the unirrigated as in the irrigated soil. Furthermore, the results show conclusively that the crops have stimulated growth of organisms which will develop on synthetic agar. Considerable of this stimulation is produced by the plant residues which are left on the cropped soil but are missing from the fallow soil.

The ammonia produced by this series is highest in the fallow and considerably lower in the oats and alfalfa plots. The quantity produced in

the potato soil is only slightly lower than the quantity produced in the fallow, while the corn shows a slight decrease below the potato soil. As has been the tendency throughout each series, the ammonifying powers decrease considerably in the fall, and this decrease is greater in the fallow than in any of the cropped soils.

Here for the first time the fallow soil has a higher nitrifying power than the alfalfa soil. This is due to the difference in moisture content of the soil, for the unirrigated alfalfa plot became very dry during the summer and fall, while the fallow remained moist throughout the year. The very high nitrifying power of the potato soil is shown again in this series, making it certain that this is not due to accident but is characteristic of the potato plot.

The influence of crop upon the bacterial activities of the soil is emphasized in the result given in Table XXXIII, in which we have the average from all the plots receiving the different amounts of water.

TABLE XXXIII.—Average bacterial activity of the soil as influenced by crop

NUMBER OF BACTERIAL COLONIES DEVELOPING IN 4 DAYS ON SYNTHETIC AGAR

Crop.	Sampled May 1.	Sampled Aug. 1.	Sampled Nov. 28.	Average.
Alfalfa.....	8, 308, 000	6, 600, 000	4, 500, 000	6, 469, 000
Oats.....	6, 392, 000	5, 492, 000	4, 349, 000	5, 411, 000
Corn.....	5, 833, 000	5, 666, 000	3, 608, 000	5, 035, 000
Potatoes.....	5, 900, 000	6, 083, 000	4, 792, 000	5, 591, 000
Fallow.....	4, 883, 000	4, 475, 000	2, 550, 000	3, 969, 000

MILLIGRAMS OF AMMONIA PRODUCED IN 100 GM. OF SOIL IN 4 DAYS

Alfalfa.....	50. 9	50. 7	40. 1	47. 2
Oats.....	51. 7	57. 8	48. 1	52. 5
Corn.....	52. 8	58. 4	45. 2	52. 1
Potatoes.....	55. 2	64. 2	47. 9	55. 7
Fallow.....	63. 9	73. 0	51. 2	62. 7

MILLIGRAMS OF NITRIC NITROGEN PRODUCED IN 100 GM. OF SOIL IN 21 DAYS

Alfalfa.....	3. 15	7. 48	3. 08	4. 56
Oats.....	2. 40	4. 00	3. 00	3. 13
Corn.....	2. 18	3. 50	1. 48	2. 38
Potatoes.....	3. 00	15. 55	5. 60	8. 04
Fallow.....	1. 30	5. 50	2. 48	3. 09

The most marked characteristic of all these results is that they are extremely low when compared with the results obtained on other soils, thus showing that the limiting factor is organic matter. The number of organisms is highest in the alfalfa and lowest in the fallow; and, with the

single exception of potato plots in the summer, this same statement holds for the nitrifying powers of the soil. These results would appear to be absolutely contrary to the findings of Heinze (24), Russell (48), and others (5, 47), who have found fallow to increase not only the number but all the bacterial activities of the soil. But it must be remembered that these investigators were working with soil which was alternately fallowed and cropped, and on this there would be left plant residues, while we have been working with a soil which has been continually fallow for 12 years, the organic matter of which has been reduced to a minimum. The results do, however, show the contentions of Hiltner to be unfounded, for the low nitrate content of alfalfa is due to the plant's rapidly removing the nitrates as formed and not due to the lack of nitrifying powers in the alfalfa soil.

#### COMPARISON OF BACTERIAL ACTIVITIES AND CROP PRODUCED ON SOIL

It is interesting to compare the nitric nitrogen found in the soil and the nitrogen removed in the crop with the various bacterial activities. In order to make these results more comparable, the average nitric nitrogen and nitrous nitrogen in the soil, the nitrogen removed in the crop, the number of organisms developing on synthetic media, the ammonia and nitric nitrogen produced in the laboratory by the fallow soil and the unirrigated soil have been taken as 100 per cent, and each of the cropped and irrigated soils compared with these. The summarized results are given in Table XXXIV.

TABLE XXXIV.—*Comparison of bacterial activities and crop produced on soil*

Crop or treatment.	Nitric nitrogen in soil.	Nitrous nitrogen in soil.	Bacterial colonies.	Ammonifying powers.	Nitrifying powers.	Nitrogen in crop.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Fallow.....	100	100	100	100	100	.....
Alfalfa.....	36	233	163	76	148	.....
Oats.....	36	167	136	85	103	.....
Corn.....	33	56	127	84	77	.....
Potatoes.....	99	122	141	90	261	.....
No water.....	100	100	100	100	100	100
15 inches.....	48	115	99	103	98	140
25 inches.....	51	62	95	96.6	98	140
37.5 inches.....	43	115	93	104	98	145

These data show that the crop has reduced the quantity of nitric nitrogen of the soil, but has increased the efficiency of the nitrifying bacteria, owing to the removal of the nitrate producer, while, on the contrary, oats and alfalfa have increased the nitrous-nitrogen content. That this is due to the compact nature of the soil is seen from the results, for the nitrous nitrogen increases as the aeration of the soil decreases and is very pronounced in the alfalfa. In the potato soil, which is cultivated, it is less than

in the fallow, which is not cultivated. The crop in every case increases the number of organisms, and this in direct relation to the plant residues left on the soil. However, we find the ammonifying powers varying in the opposite order. The nitrifying powers are increased by the alfalfa and potatoes. Hence, we can conclude that the alfalfa not only feeds closer upon the soluble nitrates of the soil but also makes a much greater drain upon the insoluble nitrogen of the soil by increasing its nitrifying powers. It therefore would deplete the soil if the entire crop be removed, more readily than would any of the other crops.

The application of water has decreased the nitric nitrogen found in the 6 feet of surface soil, but has slightly increased the nitrous nitrogen of the soil, while the number of organisms remain about the same in all the soil except those receiving 37.5 inches of water, and in these the number decreases. The ammonifying powers of the soil are slightly increased by the water, while the nitrifying powers are very uniform. But this holds only for the fall, for during the spring we obtain the following results for nitrifying powers:

	Per cent.
No water .....	100
15 inches of water .....	125
25 inches of water .....	115
37.5 inches of water .....	135

The nitrogen removed in the crop increases, but not in the same proportion as does the ammonifying and nitrifying powers. Furthermore, we have a rapid decrease in the nitric nitrogen of the soil. Especially is this true where the larger quantities of water are applied. The results therefore indicate that the effect of the excessive use of irrigation is not only a waste but the yield of the crop is depressed, and the depressed yield is due to the water carrying the soluble nitrates beyond the sphere of action of the plant roots. Furthermore, it increases the ammonifying and nitrifying powers of the soil during the spring and summer, with the result that a greater quantity of soluble plant food produced is carried out by the drain waters, and the soil is to this extent needlessly depleted of its nitrogen.

#### SUMMARY

The quantity of nitric nitrogen in the surface 6 feet of alfalfa soil is comparatively low throughout the season, but is higher in the fall than in the spring or summer. The quantity present decreases as the water applied increases; yet the quantity formed in the soil increases as the water applied increases, but is greatest per acre-inch of water when only 15 inches of water are applied.

The quantity of nitric nitrogen in the surface 6 feet of potato, oats, corn, and fallow soil decreases as the water applied increases; but the quantity formed for each of the cropped soils is greatest where the largest quantity of water was applied. The quantity formed per acre-inch of water applied is greatest where only 15 inches of water were applied.

Large quantities of nitric nitrogen disappeared from the fallow soil during the summer months. This is attributed to the growth of bacteria, which transforms it into protein substances and not to denitrification.

The larger applications of irrigation, 37.5 and 25 inches of water, carry much of the nitric nitrogen beyond the sphere of action of the plant, and this accounts for the decrease in crop yield, which is often noted when excessive quantities of irrigation waters are applied to a soil.

The application of water to a soil depresses the number of organisms which will develop upon synthetic agar in alfalfa, oats, and potato soil, but increases them in fallow. The results obtained with the corn are irregular.

The ammonifying powers of all the soil, except the alfalfa, was increased by the application of irrigation water.

Water increased the nitrifying powers of all the soils except the oat soil.

There was a difference of 2 degrees Fahrenheit in the temperature of the soil of irrigated and unirrigated in favor of the unirrigated. This difference in temperature was perceptible to a depth of 4 feet.

The number of organisms was higher in the cropped than in the fallow plots, and this is probably due to the plant residues left upon the cropped soil.

Naming the soils in the order of increasing ammonifying powers, we have alfalfa, oats, corn, potato, and fallow. By naming them in the order of increasing nitrifying powers, they are fallow, corn, oats, alfalfa, and potato.

The alfalfa not only feeds closer upon the nitric nitrogen of the soil than do other crops but it also increases the nitrifying powers of the soil. Hence, it would deplete the soil of its nitrogen more rapidly where the entire crop is removed than would other crops.

The use of irrigation water increases the bacterial activities of the soil, which render soluble the nitrogen, and where excessive quantities of water are used considerable of this is washed from the soil, thus unnecessarily depleting the soil of its nitrogen. This in turn gives diminished yields on the soil.

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## THE PINK BOLLWORM, PECTINOPHORA GOSSYPIELLA

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### INTRODUCTION

The pink bollworm (*Gelechia*) *Pectinophora gossypiella* Saunders,<sup>2</sup> is one of the most destructive cotton insects known and ranks among the half-dozen most important insect pests of the world. It occurs in the cotton districts of Asia, Africa, and the Hawaiian Islands, its ravages in these regions amounting to more than those of all other cotton insects combined. The pink bollworm repeatedly reduces the yield of lint 50 per cent or more and materially lessens the amount of

<sup>1</sup> While assuming full responsibility for the observations here recorded, the writer wishes to acknowledge that he has gathered many suggestions from the work of previous students and especially from Dr. D. T. Fullaway, who during the early part of the writer's stay in Honolulu gave him the benefit of his intimate acquaintance with the insect in the field. To Mr. C. E. Pemberton the writer is indebted for painstaking and laborious monthly examinations of many thousands of seeds, continuing after the writer's departure from Honolulu an experiment with the caterpillars in stored baled cotton for the purpose of ascertaining their longevity under such conditions. To Prof. J. F. Rock, of Honolulu, the writer is indebted for all plant identifications and for helpful information about localities of some of the rarer malvaceous plants in the Hawaiian Islands, which it was desirable to investigate as possible food plants of the pink bollworm. The identifications of the parasites are to be credited to Messrs. S. A. Rohwer, J. C. Crawford, and A. A. Girault. The writer wishes especially to acknowledge his obligations to Mr. Carl Heinrich and to Dr. Adam Böving, both of whom have given much assistance during the preparation of the systematic part of this paper. With the exception of figure C, Plate 9, which was drawn by Dr. Böving, the illustrations have all been made under the writer's direction by Mr. H. B. Bradford, and much credit is due him for his painstaking work, which greatly enhances the value of the paper.

<sup>2</sup> The species has been placed by European specialists in the genus *Gelechia*, but it is very distinct from this genus both in the imago and as larva and pupa. A new genus is here characterized for it and for the closely related *P. malvella* Zeller, the larva of which feeds in the seeds and capsules of althea and malva in Europe.

oil obtained from the seeds (10, 12, 37).<sup>1</sup> The minimum yearly loss from this insect in Egypt is estimated at 10 per cent of the value of the crop, but normally much more damage is done by it. Maxwell-Lefroy states (20) that the minimum loss in India is more than \$10,000,000 annually. In the Hawaiian Islands the cultivation of cotton has practically been abandoned on account of this pest, which during 1915 infested from 50 to 99 per cent of the bolls in the few fields yet remaining and destroyed from one-half to nine-tenths of the lint.

Similar damage would undoubtedly result if the insect by any accident should be established in the cotton areas of the United States, and it would be difficult to overestimate the importance of guarding against such introduction (32). The pest might easily prove even more serious than the cotton boll weevil, and it would certainly effect enormous annual losses.

Very fortunately this insect has not yet become established in the United States. The regulations of the Federal Horticultural Board in requiring the fumigation of all foreign cotton have reduced to a minimum the danger of its introduction from abroad. Similar precautionary measures unfortunately have not been taken in time by the neighboring Republic of Mexico, nor in South America, and it has been discovered recently that the pink bollworm has been introduced accidentally into both Mexico and Brazil within the last few years through cottonseed importations from Egypt and has become established in important cotton regions of these countries.

Up to 1912 the Brazilian cotton crop was free from any serious insect depredators. During the next two years large importations of Egyptian cottonseed were made for the purpose of improving the grades of cotton and this seed was distributed free to cotton producers without previous fumigation, with the result that the pink bollworms present in the Egyptian seed were thoroughly scattered over and established in all the cotton regions in Brazil during 1915. During the following year the pest caused a loss of 50 per cent of the cotton crop in some localities. This accidental introduction of the pink bollworm can never be remedied and will effect a perpetual diminution of the resources of Brazil. By the application of the present scientific knowledge of the insect and of the crop, cultural methods can probably be evolved and effective parasites possibly may be introduced which together will make cotton remain a profitable crop in Brazil, but the pink bollworm will continue to cause a very material reduction in the profits in spite of any measure which may be taken against it. This calamity could have been prevented by a properly enforced regulation, such as we have in the United States, covering the importation of cottonseed and requiring the fumigation of all imported seed.

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<sup>1</sup> Reference is made by number to "Literature cited," p. 366-370.

It has not been possible to ascertain how widely distributed the pest has become in Mexico beyond the Laguna district near San Pedro, where it is already a serious pest, but its presence there constitutes a very grave menace to our cotton fields. When it is considered that the introduction of this insect into American cotton fields would mean a permanent annual loss of millions of dollars to the United States, it becomes evident that all possible precautions should be taken to prevent or delay its arrival.

An essential step toward this end is a thorough knowledge of the insect in all its stages which will enable its prompt recognition in any stage, even from fragments, by the agents employed to prevent its introduction. The detailed description given in this paper provides this means of identification. It is based on an investigation of its life history and habits, conducted in the Hawaiian Islands during the summer of 1915 and subsequent anatomical studies made from material from various sources.

There is added to the paper a similar detailed descriptive and anatomical study of another lepidopterous insect, *Pyroderces rileyi* Walsingham, which may be called the "scavenger bollworm" because it frequently occurs in decayed or dried bolls injured by other insects. It seems desirable to include this supplemental study of *Pyroderces rileyi*, which frequently has been and may be mistaken for the pink bollworm. The anatomical details given in this paper will make it possible for the inspector to distinguish readily these two insects.

#### ORIGINAL HOME AND PRESENT RANGE

Although the species was first noticed and described from India, it is not probable that India is its original home.

Saunders expressed the belief, in his description of the species (1, 2), that the pink bollworm was imported into India with American cotton, which it preferred to the Indian varieties of *Gossypium*. If so introduced into India it was probably with the seed of some American variety of cotton grown in some part of Africa. Other early East Indian records have been traced by Durrant (26) to Egyptian cottonseed, and Africa is, from all the evidence on hand, apparently the original home of *P. gossypiella*. The occurrence there as well as in southern Europe of the only other known species (*P. malvella* Zeller) of the genus *Pectinophora* is in itself a strong support for the theory of African origin.

The species has spread to most, if not all, the cotton districts in Africa, Asia, Japan, Ceylon, Straits Settlements, Philippines, and Hawaiian Islands; and within the last few years it has been brought to the American continent and is already established in Brazil and Mexico (3, 4, 13, 14, 15, 20, 21, 22, 23, 25, 26).

This nearly cosmopolitan distribution leaves the United States practically the only large cotton-producing country free from the pest, and

it emphasizes the importance of maintaining for our country this enormous advantage over the rest of the world in the matter of cotton production.

#### HOW TO DISTINGUISH THE PINK BOLLWORM IN THE FIELD

Definite and final determination of *P. gossypiella* in any stage can be made only by the aid of the microscope; and, unless a collector or inspector is thoroughly familiar with the species, all suspected material should be sent at once to the Bureau of Entomology for determination. Even a fraction of the insect in any of its stages can be recognized under the microscope by the characters given in succeeding sections of this paper.

The following essential characters, all of which can be discerned by the aid of a common pocket lens, will enable the practical worker to make a reasonably certain preliminary determination of the insect in all its stages in the field.

If a small dark-brown moth is caught in the cotton field or in a cotton mill or warehouse and is found to have the forewings pointed and the hindwings broad and sinuated below the tip and to possess long curved palpi and long stiff hairs on the first antennal joint, it is reasonably certain that the moth is *P. gossypiella*, the adult of the pink bollworm (Pl. 7, A).

If, within the cotton boll or associated with stored cottonseed, a small white or pinkish caterpillar with brown head is found and under a hand lens the mandibles are seen to have four teeth (Pl. 10, D-G) and the crotches on the abdominal prolegs form a partial circle or horseshoe, opening outwards (Pl. 10, K), the caterpillar will most probably prove to be the pink bollworm.

Again, if, within a cotton boll or otherwise associated with cotton in the field or in the mill, a small lepidopterous pupa is found, which under the lens is found to be entirely covered with a short velvety pubescence and to possess a short, curved, upturned hook at the posterior end (Pl. 12, A-D), it may with considerable certainty be determined as a pupa of the pink bollworm.

#### GENERIC DESCRIPTION

**PECTINOPHORA**, new genus (Gelechiidae).

Type: *Gelechia gossypiella* Saunders.

**MOOTH.**—Face and head smooth. Labial palpi long, recurved, reaching above vertex; second joint thickened on the underside with slightly furrowed brush, which is evenly attenuated toward apex; terminal joint shorter than second, somewhat thickened with scales in front, compressed, pointed. Maxillary palpi minute, deflected. Tongue long, spiraled, scaled in its entire length. Antennæ serrated and finely ciliated on the underside; basal joint with heavy but sparse (5-6) pecten. Thorax smooth. Forewings (fig. 1, A) elongate ovate, pointed, smooth; 12 veins, 7 and 8

stalked to costa, rest separate, 1b furcate at base.<sup>1</sup> Hindwings (fig. 1, B) somewhat broader than forewings, trapezoidal; costa deflected from the middle; apex pointed; termen sinuate; 8 veins; 8 connected with cell by an oblique bar; 6 and 7 closely approximate at base; 3 and 4 connate; 5 parallel with 4; frenulum simple in the males, triple in the females. Male genitalia (Pl. 8, B), with harpes and uncus well developed; tegumen evenly chitinated. Posterior tibiae (Pl. 8, A) hairy above.

LARVA.—Head (text fig. 2 and Pl. 9) spherical, nearly circular in outline viewed from above, a little wider than long; greatest width a little behind the middle; incision of dorsal hind margin about one-fourth of the diameter of the head; distance between dorsal extremities of hind margin about one-half of the width of the head. Front triangular, reaching beyond the middle; adfrontal sutures somewhat undulating,

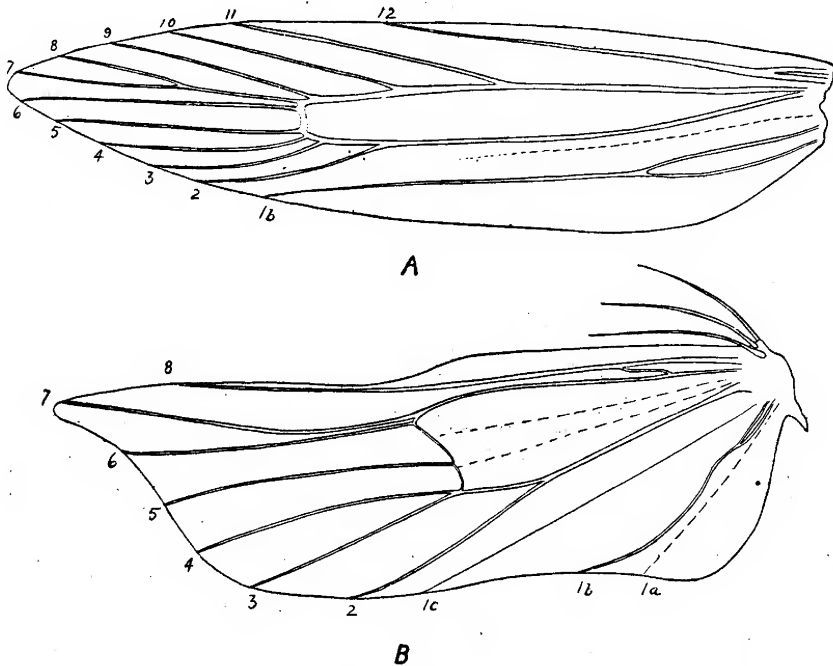


FIG. 1.—*Pectinophora gossypiella*: A, Venation of forewing; B, venation of hindwing.

reaching to the incision of hind margin; adfrontal ridges converging from near the middle, at the point of attachment of tentorial arms, to the longitudinal ridge, which is one-half as long as front. Projection of the dorsal margin over the ventral is one-half of the diameter of the head. Triangular plates of hypostoma distinctly separated by a slightly pigmented gula, nearly equilateral, but somewhat elongated and projecting slightly beyond the ventral margin of epicranium.

Ocelli six; i, ii, v, and vi forming a parallelogram; iii and iv on a line between ii and v; v smaller than the rest.<sup>2</sup> Epistoma with the usual two pairs of setae ( $E_1$ ,  $E_2$ ) well developed.

<sup>1</sup> The European (*Gelechia*) *Pectinophora malvella* Zeller exhibits an amount of variation of the venation in the forewing which is very unusual in this group of insects. Veins 2 and 3 in this species are sometimes coincident or partly coincident at base or at tip; the variations sometimes differing in the two wings of the same insect. No such variation has been ascertained in *P. gossypiella*, where the venation seems constant, as given above.

<sup>2</sup> This numbering of the eyes differs from that of Fracker in that his numbers 5 and 6 are reversed, so as to make them continuous with the rest. (Fracker, S. B. *The Classification of Lepidopterous Larvæ* . . . 169 p., 10 pl. Urbana, Ill., 1915. Bibliography, p. 145-146. Illinois Biological Monographs, v. 2, no. 1.)



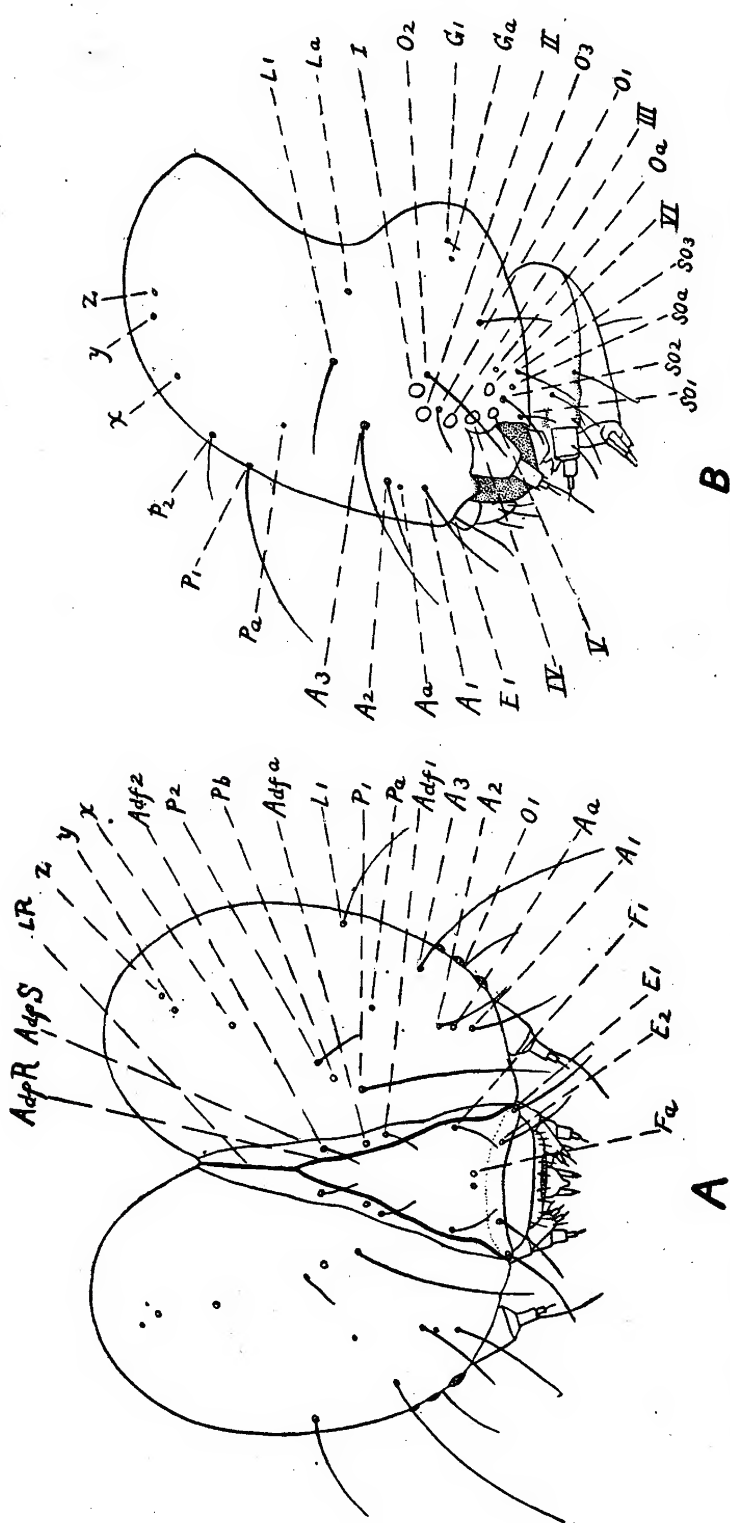


FIG. 2.—*Pectinophora gossypiella*: A, Head capsule of larva from front. B, Head capsule of larva from side. *AdfR*, adfrontal ridge; *AdfS*, adfrontal suture; *LR*, longitudinal ridge; *E<sub>1</sub>*, epistomal seta 1; *E<sub>2</sub>*, epistomal seta 2; *F<sub>a</sub>*, frontal puncture; *F<sub>1</sub>*, frontal seta 1; *Adf<sub>1</sub>*, adfrontal seta 1; *Adf<sub>2</sub>*, adfrontal seta 2; *Adf<sub>a</sub>*, adfrontal puncture; *A<sub>1</sub>*, anterior seta 1; *A<sub>2</sub>*, anterior seta 2; *A<sub>3</sub>*, anterior seta 3; *A<sub>a</sub>*, anterior puncture; *P<sub>1</sub>*, posterior seta 1; *P<sub>2</sub>*, posterior seta 2; *P<sub>a</sub>*, posterior puncture a; *P<sub>b</sub>*, posterior puncture b; *x*, *y*, and *z*, ultraposterior punctures; *L<sub>1</sub>*, lateral seta; *L<sub>a</sub>*, lateral puncture; *I*, ocellus i; *II*, ocellus ii; *III*, ocellus iii; *IV*, ocellus iv; *V*, ocellus v; *VI*, ocellus vi; *O<sub>1</sub>*, ocellar seta 1; *O<sub>2</sub>*, ocellar seta 2; *O<sub>3</sub>*, ocellar seta 3; *O<sub>a</sub>*, ocellar puncture; *SO<sub>1</sub>*, subocellar seta 1; *SO<sub>2</sub>*, subocellar seta 2; *SO<sub>a</sub>*, subocellar puncture; *G<sub>1</sub>*, genal seta; *G<sub>a</sub>*, genal puncture.

Frontal punctures (Fa) close together, anterior to frontal setæ ( $F_1$ ); distance between punctures less than distance between puncture (Fa) and setæ ( $F_1$ ); frontal setæ ( $F_1$ ) and adfrontal setæ ( $Adf_1$  and  $Adf_2$ ) nearly equidistant; second adfrontal seta ( $Adf_2$ ) approximate to but before beginning of longitudinal ridge (LR); adfrontal puncture ( $Adfa$ ) midway between adfrontal setæ.

Epicranium with normal number of primary setæ, 13, and punctures, 7, and with three small ultraposterior punctures,<sup>1</sup> (x, y, and z).<sup>2</sup>

Anterior setæ<sup>3</sup> ( $A_1$ ,  $A_2$ ,  $A_3$ ) in a slightly obtuse angle;  $A_1$  and  $A_2$  closer together than  $A_2$  and  $A_3$ ; anterior puncture (Aa) between  $A_1$  and  $A_2$ . Posterior setæ<sup>4</sup> ( $P_1$ ,  $P_2$ ) and posterior punctures (Pa, Pb) near the middle of the head;  $P_1$  on the level with adfrontal puncture<sup>5</sup>;  $P_2$  posterior to  $Adf_2$ . Pa equidistant from  $P_1$ ,  $A_3$  and the lateral seta ( $L_1$ ), remote from anterior group, nearly on the level with  $P_1$ ; lateral seta ( $L_1$ ) remote from  $A_3$ , nearly on the level of Pb; lateral puncture (La) posteroventral to the seta, remote. Of the ocellar setæ ( $O_1$ ,  $O_2$ ,  $O_3$ ),<sup>6</sup>  $O_1$  is equidistant from and lateral to ocelli ii and iii,  $O_2$  is closely approximate and posteroventral to ocellus i;  $O_3$  is directly ventral and remote from  $O_2$ , on a line with ocelli v and vi; ocellar puncture (Oa) between  $O_3$  and ocellus vi, approximate to latter. Subocellar setæ ( $So_1$ ,  $So_2$ ,  $So_3$ ) triangularly placed, nearly equidistant; subocellar puncture (Soa) between and equidistant from  $So_2$  and  $So_3$ . Genal seta ( $G_1$ ) and puncture (Ga) both present; puncture anterior to seta.

Labrum (Pl. 10, I, J) with median incision rather deep and evenly rounded. The three lateral setæ ( $La_1$ ,  $La_2$ ,  $La_3$ ) close to edge,  $La_1$  and  $La_2$  closely approximate,  $La_3$  remote; median setæ ( $M_1$ ,  $M_2$ ,  $M_3$ ) in the usual Micro arrangement with  $M_2$  lateral and slightly posterior to  $M_1$ ;  $M_3$  close to anterior margin on a line with  $La_3$ ;  $M_1$  and  $M_2$  on a line respectively with  $La_2$  and  $La_1$ .

Epipharyngeal shield (ES) merely a slight chitinization of the edge of the median incision; epipharyngeal setæ narrow plates, triangularly grouped near anterior margin. Epipharyngeal rods not discernible in the labrum proper, only represented by their posterior projections, which are rather well developed.

Mandibles (Pl. 10, D-G) strong, as broad as long, with four stout, rather short teeth; the three lower ones pointed; the upper blunt; a fifth lower tooth is slightly indicated on the underside; one long and one shorter seta on upper side near lower edge.

Labium and maxillæ normal (Pl. 9, C).

Antennæ (Pl. 10, H) four-jointed, with second joint considerably longer than joint 3, longer than broad; the longer seta longer than the entire antenna; papillæ minute, much shorter than third joint.

Three pairs of normal thoracic feet; four pairs of abdominal prolegs with crotches of uniform size in an incomplete circle, opening outwardly (Pl. 10, K); anal prolegs with a transverse row of uniordinal hooks.

The arrangement of the body setæ is normal, as shown in Plate 11, A, B. It differs from that of *Gelechia* in having the three setæ on prespiracular plate of prothorax nearly equidistant, while in *Gelechia* the posterior seta is farther separated from the two others than they are from each other, and in having the three setæ vii of the proleg-bearing abdominal segments arranged in a triangle, not in a line as in *Gelechia*.

<sup>1</sup> The nomenclature of the head setæ has been adopted from Heinrich (39) with certain minor modifications, noted in the following footnotes and concurred in by Mr. Heinrich.

<sup>2</sup> So-called "secondary punctures" of Heinrich, sometimes bearing minute setæ.

<sup>3</sup> Anterodorsal setæ of Heinrich.

<sup>4</sup> Posterodorsal setæ of Heinrich.

<sup>5</sup> The term "on the level with" is used in these descriptions as the head setæ are seen in frontal projection (fig. 2, A); anything above a level is termed "posterior" and anything below is termed "anterior."

<sup>6</sup> Heinrich's numbering reversed.

The genus differs further from *Gelechia* in the possession of an antennal pecten in the moth, and in the arrangement of the setæ of the larval head;  $Aa$  is anterior to  $A_2$ , not posterior to it as in *Gelechia*;  $P_1$  and  $P_2$  are posterior respectively to  $Adf_1$  and  $Adf_2$ , which in *Gelechia* are nearly opposite to these, and  $L_1$  is posterior to  $P_1$ , not on the level with it as in *Gelechia*.

The most striking larval difference is in the crotches of the abdominal prolegs, which are uniordinal and arranged in an incomplete circle, broken outwardly (Pl. 10, K). In *Gelechia* they are biordinal and in a complete circle.

PUPA.—The pupa of *Pectinophora gossypiella* is pubescent, without any long setæ except on last joint, and thus is easily distinguished from the smooth, seta-bearing pupa of *Gelechia*; cremaster present.

#### SPECIFIC DESCRIPTION

MOTH (Pl. 7, A).—Labial palpi reddish brown; second joint with two diffused black bars exteriorly; terminal joint with two well-defined, broad, black annulations, one at base, the other at apical fourth. Antennæ brown with narrow black annulations; basal joint with long black pecten. Face and head light reddish brown with some pale iridescent scales. Thorax reddish brown with a sprinkling of black around the collar; patagia somewhat lighter brown, unmottled. Forewings darker brown with a series of small, ill-defined, black spots along the costal edge from base to apical fourth, where there is a larger dash of light ochreous brown; dorsal edge and apical part of wing suffused with darker, blackish brown; the middle of the wing is irregularly sprinkled with blackish scales and contains on the cell an ill-defined, round, blackish spot, sometimes divided into an upper and lower spot; there is also a smaller spot on the base of the cell; the pattern of the wing is rather vague and there is considerable variation in different specimens; in many there is an ill-defined blackish fascia at apical fourth just before the light costal dash, but in other specimens this fascia is not present and the round dorsal spot is dissolved into several smaller spots. Cilia light ochreous brown, streaked with blackish. Hindwings dark fuscous, somewhat iridescent, lightest towards base; cilia ochreous, terminal and apical parts suffused with dark fuscous: vein 1c with long, ochreous fuscous hairs on the upper side. Abdomen flattened and ochreous above, dark brown laterally with underside suffused with black and with ochreous scaling at the joints. Legs (Pl. 8, A) blackish fuscous with narrow ochreous annulations at the joints. The abdomen is very similarly shaped in the male and in the female and it is exceedingly difficult to distinguish the sexes, even in living moths, without dissection or by examination of the frenulum. The male genitalia (Pl. 8, B) are remarkably small in proportion to the size of the species: harpes narrow at base, broadening towards tip; tip strongly haired; a cluster of long, heavy, straight spines from inner side, well within the tip; sacculus armed on its edge with a row of stout spines; uncus moderately long, broad at base, tapering to a point, laterally heavily haired; ædœagus short, stout, with a terminal hook. In the female the ovipositor is weakly chitinized, covered with stiff hairs; genital plate heart shaped; bursa copulatrix with two opposite, strongly chitinized, hornlike, serrated invaginations (Pl. 8, C).

Alar expanse 15 to 20 mm.

FULL-GROWN LARVA.—The full-grown larva (Pl. 11, A) is 11 to 13 mm. long, cylindrical, white, with dorsal side strongly suffused with pink. Head reddish brown with blackish brown mandibles and the other trophi yellowish. Thoracic shield rather small, divided in the middle, dark brown. Anal plate small, dark brown. Tubercles small, but distinct, yellowish brown, surrounded by deeper pink than the prevalent suffusion and bearing rather short, dark-brown setæ. Crotches of abdominal feet 15 to 17.

**PUPA.**—The pupa (Pl. 12, A-D) is 8 to 10 mm. long, rather plump, reddish brown; posterior end pointed and terminating in a short, stout, upwardly turned hooklike cremaster; entire surface finely pubescent; no long setæ, spines or hooks, except on last joint; fronto-clypeal suture distinct and curved sharply upward; clypeus, labrum, pupal eyes and mandibles distinctly indicated; antennæ diverging at their extreme tip and not reaching to the tips of the wings; metathoracic legs reaching slightly beyond the wings to fifth abdominal segment. Spiracles small, normal. Anal opening large, slitlike, surrounded by strong hooked setæ, 5 or 6 on each side; cremaster surrounded with 6 to 8 similar, strong, hooked setæ. Genital opening slitlike, single in both sexes. When mature, the pupa becomes much darker (Pl. 12, C); the imago's eyes can be seen prominently under the gena of the pupal skin, and the segmentation of the adult antennæ and legs becomes discernible.<sup>1</sup>

**EGG.**—Elongate oval, flattened; about 1 mm. long and 0.5 mm. broad; the shell is pearly white, with a finely wrinkled surface.<sup>2</sup> When newly laid, the egg has a slightly greenish tint. At maturity it turns reddish.

### SEASONAL HISTORY AND NUMBER OF GENERATIONS<sup>3</sup>

The small eggs are difficult to detect without the aid of a lens. They are laid singly or in small groups on any part of the green cotton boll or its calyx or even in the flower, but are by far most commonly found near the apex of the green boll in the slight longitudinal depressions which indicate its divisions (fig. 3, a). From 1 to 4 eggs are commonly seen, and sometimes as many as 20 may be found on a single boll, probably laid by several females. The number of eggs laid by a single female is difficult to ascertain in nature, but dissections prove that each female is capable of laying more than 100 eggs. The egg hatches in from 4 to 12 days after it is laid.

The larva when first hatched is very small, glassy white, with light-brown head and thoracic shield. It tunnels into the boll under the egg-shell or near by and feeds in the beginning on the soft inner walls or in the equally soft partitions separating the divisions of the boll. The larva is easily overlooked at this stage when the boll is opened and

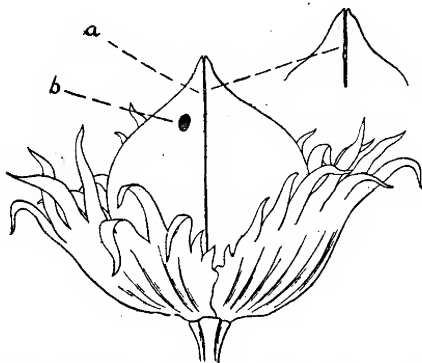


FIG. 3.—Cotton boll infested with *Pectinophora gossypiella* a, Eggs in situ; b, exit hole of moth. (Original.)

<sup>1</sup> The terminology of the pupa has been adopted from Miss Edna Mosher's valuable paper: (Mosher, Edna. A classification of the Lepidoptera based on Characters of the pupa. In Bul. Ill. State Lab. Nat. Hist., v. 12, art. 2, p. 17-159, pl. 19-27. 1916.)

<sup>2</sup> The egg has been described by Fullaway (16, p. 18, fig. 10) as having "a peculiar sculpturing of the surface, which renders them unmistakable when observed with a hand lens," and his figure shows a regular cross-line effect, but there is no such true sculpture of the egg surface. When laid, the egg is soft and smooth and the surface merely becomes irregularly wrinkled shortly after it is laid.

<sup>3</sup> The biological observations on which this paper is based were made by the writer in 1915 in Honolulu, T. H.; but they coincide with the results of other students of the insect in other parts of the world, and the data will undoubtedly hold true, with slight modifications, in the United States, if the pest becomes established here.



examined; but the minute entrance hole with a small amount of pellets of reddish frass and the empty shell of the egg are sure indications of infestation, and dissection of the boll will reveal the small larva mining within the wall.

Infested bolls normally become more or less recognizable by discoloration of the shell, which soon assumes a reddish or black color over the infestation; but as such discoloration may occur in uninfested bolls from other injury and does not always follow infestation of the pink bollworm, no conclusive discrimination between infested and uninfested bolls can be made without the discovery of the eggshell and the entrance hole.

There is considerable individual variation in the further course of the attack, partly depending upon the location of the egg and the condition of the boll and partly upon the direction the larva may happen to choose. Most commonly the larva bores in near the apex of the boll and tunnels down through the walls to the base before it attacks one of the lowest seeds. This it eats partially and then proceeds to the next seed above, ending as a full-grown larva in one of the seeds nearest the tip of the boll. Sometimes, however, the opposite movement takes place. A larva generally confines itself to a single section of the boll, but an adjoining section is often invaded, and sometimes all sections will be more or less attacked by a single larva.

If a boll for any reason becomes unsuitable, the larva will readily leave it and migrate to another, into which it eats its way through the shell, making a large, conspicuous, frass-surrounded hole.

The larva eats the seeds and tunnels and soils the lint, causing the arrest of the growth and the rotting or premature and imperfect opening of the boll. Not only the seeds and the lint actually attacked are lost, but the uninfested parts of the boll are retarded in growth and greatly depreciated in value by the attack of even a single larva. When, as is often the case, two, three, or more larvæ infest a single boll, the value of the seeds and lint is entirely destroyed.

The larva is exclusively an inside feeder within the boll and does not attack the leaves or shoots of cotton. Sometimes the young larvæ may be found in the ovary of the flower, devouring the tender ovules and preventing the formation of the lint. Such larvæ rarely attain their full growth in the flower, but migrate to a boll for their later support. Much more commonly, however, it is the larger, well-formed boll, which is attacked.

There are four larval instars. The younger larvæ are nearly pure white, with a brown head, thoracic shield, and tubercles. It is only in its last stage that the larva assumes the strong pink suffusion which has caused its popular name "pink bollworm."<sup>1</sup>

<sup>1</sup> It should be noted that the larvæ of many other Microlepidoptera assume a similar red or pinkish coloration at maturity and that the larva of *Pyroderces rileyi*, described in the latter part of this paper, which also occurs in cotton bolls, has a decided reddish color throughout its life.



The larval stage from the hatching of the egg to the spinning of the cocoon occupies from 20 to 30 days during the summer. During the colder months, or under abnormal, dry conditions, the larval period may be much prolonged. The species overwinters as larva within the seeds.

The larva normally makes its cocoon and pupates within the boll, partly within the last seed attacked. Before finishing the cocoon the larva gnaws a round hole through the outer wall of the boll to insure free exit for the issuing moth (fig. 3, b). It has an evident preference for a firm cover for its cocoon and a firm support for the imago to issue from. The cocoon is always spun next to the shell of the boll, and the exit hole is invariably gnawed through the shell, which at that time is often woody and hard, although an easier and more abundant exit surface could be found in all other directions through the loose lint of the boll, which by this time has opened.

The cocoon consists of a single thin, but rather tough, layer of dirty-brown silk. If disturbed at the time of maturity the larva may leave the boll, fall to the ground, and spin its cocoon an inch or more down in the soil or in any convenient shelter under a stone or among brush and leaves and will successfully finish its transformation. Under normal conditions in the field, however, the pupation nearly always takes place within the boll.

The pupal period lasts from 10 to 20 days. The empty pupal shell remains within the cocoon when the moth issues.

The imago is a small, inconspicuous, sluggish moth, rarely seen in nature, because it hides away during the day, mostly on the ground under stones or in brush, sometimes actually burrowing into the surface of the soil. The time of flight is from 6.30 to 8 p. m.; but, though the moths have ample wings for a strong, sustaining flight, they fly only to the nearest cotton bolls for copulation and egg laying, which under normal conditions takes place soon after issue.

The moths die shortly after oviposition. Under most favorable conditions, in a cool place supplied with water, some moths were kept alive for 32 days, but the majority died even under these conditions in from 14 to 20 days.

The entire life from the laying of the egg to the next egg laying may be accomplished under favorable conditions in 35 days, but 40 to 50 days is the more common period even in midsummer, and in the colder months the life cycle may extend over three or four months. Thus, four or five or even six overlapping generations may be produced in a year.

The winter is passed in the larva stage in the seed.

The writer's observations in Honolulu in 1915 began on May 18. At that date the insect was found in all stages; eggs, larvæ, and pupæ were obtained in the field, and one moth issued from one of the collected pupæ the following morning. About 50 per cent of the green bolls were infested at this date.

Throughout the following five months eggs, larvæ, and pupæ were collected and moths issued in the rearing jars every day. By September the percentage of infested bolls was 90 to 99 in the different fields under observation.

#### HABITS OF THE IMAGO

The imago of the pink bollworm is an inconspicuously colored moth and is very rarely observed in nature. The inconspicuousness, however, is due as much to the retired habits of the moth as to its color and is paralleled in many other Microlepidoptera. Such a common insect, for example, as the codling moth (*Carpocapsa*) *Laspeyresia pomonella*, is seldom or never observed in nature.

It is very perplexing to walk through a heavily infested cotton field and not to be able to discover a single moth, although one knows that thousands of them have issued that morning and other thousands every day for a week and that all these thousands must be somewhere near you.

The moths find their resting places during the day near or on the ground, in rubbish around the roots of the plants, or under stones. They often partially burrow into the surface of the ground for shade and concealment. Only occasionally is a specimen found on the cotton, hidden away at the base of the boll, under the large calyx.

As an experiment, several dozen moths which had issued in the rearing cages were repeatedly liberated in the middle of a cotton field by shaking them out of a jar onto the ground. Within a minute none were in plain sight. All had effectively hidden away, mostly on the uneven surface of the ground.

The same secretive habit prevails under artificial conditions indoors. Hundreds of moths were reared weekly and liberated in a small rearing house, yet rarely were any in sight after a few hours. Two hundred moths were liberated daily in a living room on seven successive days, but only by search were any to be found during the daytime.

#### REACTION TO LIGHT

Like most of its relatives, the cotton moth is negatively heliotropic and invariably seeks protection from direct sunlight and even from diffused daylight. Its time of activity is, as before stated, at dusk, from 6.30 to 8 p. m. Of a hundred or more newly issued moths liberated daily in the rearing house, a large percentage would, on the opening of the rearing jars, fly to the screened north wall of the house in an effort to escape, but shortly afterward they would be found to have left the light-exposed screens and to have crawled or flown to darker parts of the house, especially to the corners near the floor.

Nor is artificial light an attraction to the moths of this species, as it is to a large number of other Microlepidoptera. Strong kerosene and acety-

lene lamps, placed in a most effective manner, with white sheets as backgrounds, on a porch and in the windows of a cottage surrounded, within 20 feet, by heavily infested cotton fields, failed to attract a single individual of *P. gossypiella* during many evenings and nights, though efforts were made to disturb and dislodge them in the fields by beating and shaking the cotton bushes.

The trapping of these moths by light has been recorded and recommended and special lantern traps, which were believed to be effective, have even been constructed and figured (5, 8, 18, 19, 27, 30); but this method of combating the pest is certainly futile. The idea that these moths were attracted to light is based on very unsatisfactory evidence and is probably due to misidentification of the material collected in the traps.

The cotton fields abound in Microlepidoptera. In Honolulu the leaf-folder *Tortrix postvittana* Walker and the scavengers *Cryptoblabes aliena* and *Opogona aurisquamosa* Butler are common in the cotton fields and are of about the same size as *P. gossypiella*. These species are attracted to light, and it has probably been specimens of these and other species which were captured in the traps.

In order to study the behavior of the moth indoors and to verify, if possible, the records of their attraction to light under such conditions, a large number (200 a day for a week) of freshly emerged moths were liberated in a large living room in Honolulu. During the day none of these moths were noticeable, except when searched for in the dark corners or accidentally disturbed by the movement of a curtain or a towel. At 6.30 p. m. all these moths would come to the windows, seeking their way outdoors, and would remain motionless on the screens or curtains until daylight next morning. Then they would fly back to the dark corners of the room. If in the evening an electric lamp was lighted near the window, some of the many moths in the immediate vicinity would be disturbed and for a short time would be blinded by the strong light and would even settle on or near the lamp; but as many or more would fly away. Only very rarely would a cotton moth come within the glare of an acetylene hand lamp carried at night in the cotton field; but, if it did, it would invariably endeavor to fly away from the light.

From very many varied and repeated observations under different conditions it may be definitely stated, notwithstanding the many other statements to the contrary, that *Pectinophora gossypiella* is not attracted to light, but is, on the contrary, shy of all light, natural and artificial.<sup>1</sup>

It has been suggested that a darkened space might be a barrier which the moths would not fly into or through. If such was the case, it might have a practical bearing in the prevention of the escape of the moths from cotton mills; but this is not true. The tendency of the moths, on the contrary, is to fly into such a dark space.

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<sup>1</sup> Vosseler (7, p. 407) and Stuhlmann (11, p. 216) have reached the same result.

A small cottage of five rooms (fig. 4) on one floor was utilized in testing the behavior of the moths in relation to light and darkness. All of the rooms opened out on a narrow, central passageway (E), 15 feet long, which could be made the darkest part of the house by closing up the main entrance. Several hundred moths were liberated on different days in the rooms. A large percentage (nearly half) could always be found after a few hours in the dark passageway. At 6.30 p. m., if the

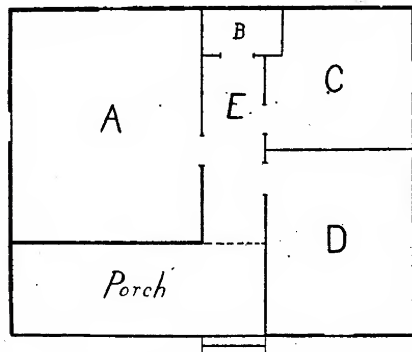


FIG. 4.—Plan of the house used for observations on the behavior of the moths of the pink bollworm in relation to light. (Original.)

front entrance was closed, these moths would leave the passageway and come to the screened windows of the rooms, seeking exit to the outdoors. Two hundred to three hundred moths, liberated one afternoon in a small darkened room (B) at the end of the dark passageway at 6.30 p. m., flew through the length of this 15-foot passageway out into the open air; nor were they hindered in doing this when the porch outside was lighted by a strong light. It is noteworthy that not a single moth was attracted to the light on the porch,

but that all flew right out through the light space to the cotton fields beyond.

It is apparent, therefore, that neither a darkened space nor a sheet of light is an effective barrier to the flight of these moths.

#### LONGEVITY OF THE LARVA AND BEHAVIOR UNDER ARTIFICIAL CONDITIONS

As already stated, the larval life of *P. gossypiella* is accomplished under normal summer conditions in from 20 to 30 days, but if the young larva is confined to dry and hard cotton seeds, either artificially or, as may happen, in prematurely ripening bolls in the field, it will live much longer. Half-grown larvæ kept in dry cotton seeds in a cool place from June to September eventually reached maturity, pupated, and issued successfully as moths. The life of the hibernating larva is normally longer, from 3 to 5 months; but these also may live for a much longer period under dry conditions unfavorable for the issue of the imago. Heavily infested, unginned seeds were baled under strong pressure into small trial bales (24 by 12 by 18 inches) in September, 1915, and placed in dry rearing boxes indoors in Honolulu. One of these bales was examined every month afterwards, and numbers of live healthy larvæ were found on each examination up to March, 1917. It was thus actually proved that the suspension of larval life may extend over 18 months; and there is no doubt, from observations of other seed-feeding lepidopterous larvæ, that the



pink bollworm may keep alive and be capable of eventual maturity even longer than this.<sup>1</sup> This ability of the larva to sustain life within the seed for a prolonged period has an important bearing on the spread of the species, as the larvæ may be transported any distance with the seed and may transform successfully and produce imagoes capable of reproduction whenever conditions become favorable.

This possible suspension of the larval life renders of uncertain value any storing of the seed as a safeguard against infestation from such seed.<sup>2</sup>

The instinct of the larva to provide a safe exit to the outer world for the moth in the field by gnawing a hole through the husk of the boll, preliminary to the spinning of its cocoon, governs the larva also under artificial conditions. If an infested boll is wrapped in tissue paper or in cloth, the larva will bite its exit hole through these additional layers, spinning its cocoon within the hole. Also, if a mature larva is confined in a small pill box or in a capsule, it will tunnel a hole through to the outer world and then, conditions being otherwise suitable, will spin its cocoon within the hole. When green infested bolls are inclosed in a sack, such larvæ as are ready to pupate will leave the bolls and eat their way out through the sack to find suitable places outside for their cocoons. Even a heavy canvas or khaki bag is no barrier. If the larva matures within a bale of dry cotton and is sufficiently near the surface of the bale to be able to work through the packed cotton, it will do so and cut its way through the covering of the bale. Such a larva will not normally make its cocoon within the hole in the sack or in the covering of the bale, but will seek a suitable place outside because of its instinct to find a firm support for the issuing moth.

This tendency in the larva to seek free access to the outer world for the issuing imago has an important bearing on the precautions necessary to insure against the introduction of the pest into the United States with imported baled cotton. It renders absolutely valueless as a preventive any burlap covering of the bales. Such covering will in no degree lessen the possibility of importation, as any larva which may be in the bale and which is able to come to the surface can easily cut through the covering, and will invariably do so. Such larvæ as are within seeds deeper in the bale will remain quiescent until the bale is opened and the pressure relieved, when they will issue quickly from the seeds and complete their transformation.

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<sup>1</sup> Gough (36) found larvæ hibernating over two years in Egypt.

<sup>2</sup> This applies particularly to seeds stored under usual conditions in a temperate climate—that is, within the bolls and in cool dark rooms unsuitable for the issuing of the imago. In the warm climate of Honolulu, however, it was found that loosely stored cotton seeds in open bins would be entirely free of larvæ in the course of a few months, contrary to the results obtained with baled cotton.

Vosseler (7, p. 407) also records an experiment with infested seeds placed on a sheet in the sun in which all the larvæ left the seeds in a few hours.



## MANNER OF DISPERSION

While *P. gossypiella* has ample wing power for an insect of its size and is capable of strong, darting flight, it is not mainly by flight that the distribution of the species to new fields is effected. The moths are rather too sluggish for sustained flight, and it is only fields near by or actually adjoining that are infested in this way.

A group of a dozen cotton plants growing only some 4,000 to 5,000 feet from the heavily infested cotton field under special observation was found well infested in early June. All the bolls, squares, and flowers were removed from these 12 plants, and the new crop of bolls, maturing in September and October, remained entirely free from infestation, although thousands of moths were liberated during the summer from the breeding house, situated some 4,000 to 5,000 feet away from them and in the opposite direction from the infested field.

Single cotton trees, grown as ornaments in gardens in different parts of Honolulu, were found entirely free from the pest throughout the summer, though others a few blocks away were heavily infested.

Nor does the wind ordinarily play any considerable rôle in the dispersion of the pest. The moths normally remain quiet in any strong breeze and, if accidentally dislodged by the wind, drop to the ground as soon as possible. Specimens were repeatedly shaken out of rearing jars in strong winds. Such specimens would be carried with the wind for a short distance, but would invariably soon settle down on the ground. The dispersion of the species by the wind, however, can not be disregarded. Under favorable conditions it is possible for both the moths and the larvæ which happen to be in loose detached cotton lint to be carried by a strong wind for a considerable distance from field to field.

By far the most important agent in the distribution of the pest is man. Owing to the possible suspension of the larval life for a prolonged period, hibernating larvæ may be transported to any distance within the seed of cotton and in due time produce adults. It is in this manner that the species has become distributed with cotton importations over such large areas and from continent to continent.

## FOOD PLANTS

The writer, from his observations in the Hawaiian Islands, is convinced that the pink bollworm is confined to the genus *Gossypium*. Statements that it feeds also in various malvaceous and other plants are probably due to wrong determinations of the insect.

Maxwell-Lefroy states (9) that the species feeds in species of *Hibiscus* in India; Dudgeon (28) records it from pomegranate in Egypt; Fullaway (16) has reported it from milo (*Thespesia populnea*) in Hawaii.

This last record, however, was based on the rearing of a single specimen from a fallen fruit of milo on the ground of the Agricultural Experiment

Station in Honolulu, and Dr. Fullaway agrees that this might well have been from a stray mature larva which had accidentally crawled into a cracked milo fruit for pupation. In order to test the record, the writer collected fruits in all stages from the milo trees on the Experiment Station grounds as well as elsewhere during the summer of 1915, examining many hundred fruits and keeping as many more in cages. Not a single pink bollworm was found in or reared from these fruits, although from 50 to 500 reared moths were liberated every week during the summer in and under the trees in an effort to have them oviposit there.

Similar observations were made with the fruits of other malvaceous plants, particularly the hau (*Pariti tiliaceum*) and the wild hibiscus (*Hibiscus arnottianus*). Large lots of the fruits were collected and examined for larvæ, and other lots were placed in rearing jars in an effort to substantiate the statements that they are food plants of the pink bollworm, but not a single specimen was either reared from or found in these fruits. The fruits of hibiscus in Hawaii harbor a microlepidopterous larva about the same size as the pink bollworm, and this is presumably the foundation for the statement in Hawaii; but these larvæ belong to a different insect, (*Crocidocema*) *Eucosma marcidellus* Walsingham.

The pink bollworm, on the other hand, was most unexpectedly found in and reared from *Gossypium tomentosum*, the small dry fruits of which seem quite unsuitable for the species. This species of *Gossypium* is indigenous to the Hawaiian Islands.

Among the several varieties of cultivated cotton, the pink bollworm seems to have no choice. The perennial Caravonica cotton is by far the most commonly cultivated variety in the Hawaiian Islands; but the bollworm attacked just as readily plots of Chinese, Sea Island, and American Upland cotton growing in the Agricultural Experiment Station grounds in Honolulu.

#### . PARASITES

The larva of *P. gossypiella* is so effectively protected within the green boll that no parasite at present found in the Hawaiian Islands can reach it as long as the shell is intact. It is only after the boll has opened or after the larva has bored its exit hole through the husk that parasites can gain access to it.

The following five species of hymenopterous parasites were reared in Honolulu during the summer of 1915 from *P. gossypiella*, but none of these is an effective check on the pest, and, all combined, they do not infest more than a small percentage: *Chalcis obscurata* Walker; *Stomatoceras pertorvus* Girault; *Pimpla* (*Itoplectes*) *hawaiiensis* Cameron; *Chelonus blackburnii* Cameron; *Parisierola emigrata*<sup>1</sup> Rohwer.

<sup>1</sup> A closely allied species, *Parisierola nigrifemur* Ashmead, is parasitic on the pink bollworm in Brazil. Maxwell-Lefroy (17) records *Apanteles depressariae* as parasitic on *P. gossypiella* in India, and Willcocks records (33) *Pimpla* sp. and (34) a braconid parasite from Egypt.

The first four of these species are parasites of the pupa. These species have other hosts besides *P. gossypiella*, and they play practically no rôle in the reduction of the species, since altogether they do not kill more than a fraction of 1 per cent.

The last-named bethylid, *Parisierola emigrata*, is an external parasite of the full-grown larva. The female works her way either through the exit hole of the cotton worm or through the lint of the opened boll into the cell in which the larva is preparing to pupate. She jumps upon the larva and paralyzes it by inserting her sting into the nervous system of the caterpillar, usually just behind the last thoracic legs. When the parasite has assured itself by biting and pulling the body of the larva that the paralyzation is effective, it deposits its eggs. These are rather large, glassy white, and normally placed one on a segment, in two rows on the underside of the caterpillar. Four to six eggs are most commonly laid on one host larva, but as many as seventeen were laid in captivity, and repeatedly eight to twelve could be found in the field. These eggs hatch within 24 hours, and the parasitic larvæ grow quickly and form a rosette on the shriveling body of the host. They become full grown in two or three days and then spin their cocoons near the host larva. The spinning of the cocoon occupies nearly two days, and before it is completed the larva voids a large fluid excrement through an opening left in the as-yet-unfinished cocoon. These excrements harden into a characteristic bifurcated black substance, which often serves to glue the cocoon to the supporting surface.

When there have been many parasites (8 to 17) on a single caterpillar, their cocoons are flimsy and white; but, when only four to six parasites have found nourishment in a single larva, they average larger in size and their cocoons are more substantial and are brownish in color.

The pupa of the parasite is at first white, with coral-red eyes, but it turns blackish within a few days. The parasite issues in from 10 to 15 days after the egg is laid.

It may be mentioned that a very large percentage of these parasites are females, about 30 to 1 male, and that parthenogenesis was repeatedly observed—seemed in fact to be the normal condition. Four generations consisting exclusively of females were produced in one experiment from a single unfertilized female.<sup>1</sup>

This bethylid was first recorded from Hawaii in 1912 and had been introduced only shortly before that time, probably from the United States. It is found rather commonly in all cotton fields on Oahu and in the Kona district, Hawaii, and it is the only parasite of *P. gossypiella* of any importance at present in the Hawaiian Islands. It is by no means an effective check, however, and destroys only 1 to 4 per cent of the larvæ.

<sup>1</sup> The life history of this species was published by the writer (40) and notes on the species by Fullaway (29).

More effective parasites of *P. gossypiella* might be expected to be found in the original home of the species, Africa, but no record of such has been made.

It is also possible that the congeneric species, *P. malvella*, found in Europe and Africa, or any other lepidopterous species with similar biology, may be preyed upon by parasites which could become of value against the pink bollworm.

An egg parasite would be by far the most promising for effective results, first, because the egg is the only stage of the species which is easily reached by a parasite, and, secondly, because the insect is thus destroyed before it has had a chance to do damage. As soon as the larva has bored into the boll, it is reasonably safe from parasites, and, even if killed, has already done serious damage to the boll. *Trichogramma minuta* Riley, which is parasitic on the eggs of the codling moth in this country, or any other species parasitic on singly laid, exposed eggs of Microlepidoptera, would be worthy of trial.

#### OTHER NATURAL ENEMIES

There are no predacious insects playing any rôle in the reduction of *P. gossypiella* in the Hawaiian Islands, and it is doubtful whether any such insect could be of economic value. The larvæ within the bolls are quite safe from them; and the numbers of moths which, for example, a mantis could catch would be quite negligible.

Large numbers of the predacious mite *Pediculoides ventricosus* Newport were found in a few instances in cotton bolls in Honolulu, and they had invariably killed the *P. gossypiella* larvæ present. This mite has also been received from Brazil with the remains of larvæ of *P. gossypiella* which it had destroyed. It is also recorded as an enemy of the pink bollworm in Egypt (33), but it seems doubtful that it can ever be really effective in the field against *P. gossypiella*. If in exceptional cases it should become sufficiently numerous to be a check on the pink bollworm, its presence would probably be so obnoxious to the workers in the field as to counteract its value as a parasite. In stored cotton this predator might readily have some effect in killing off the hibernating larvæ, but the same results may be obtained in a quicker and surer way by fumigation, and it is not believed that *Pediculoides ventricosus* can be successfully employed in any organized fight against the cotton pest.

There are very few wild birds in the cotton fields of the Hawaiian Islands, and they play no rôle in keeping the pest in check; but domestic fowls are of some benefit. In one cotton plantation on the Island of Oahu a large number of chickens, ducks, and turkeys had free access to certain inclosed areas of cotton. These birds materially assisted in the reduction of the pest by eating a large number of the moths and such larvæ as accidentally fell to the ground.<sup>1</sup>

<sup>1</sup> Vosseler (7) records that chickens and ducks voraciously picked up *P. gossypiella* larvæ which made their escape from the bolls placed on a sheet in strong direct sunlight.



Opportunity was not afforded to test this means as thoroughly as would have been desirable, by having separate plots of cotton with poultry compared with sufficiently distant control plots without poultry; but even without such careful tests it was evident that considerable protection was afforded by the poultry. Plots to which the fowls had access were less infested than adjoining plots from which they were excluded.<sup>1</sup>

#### SYNONYMY OF PECTINOPHORA GOSSYPIELLA SAUNDERS

*Depressaria gossypiella* Saunders (1).  
*Gelechia gossypiella* Meyrick (6).

*Gelechia gossypiella* Walsingham (13).  
*Gelechia gossypiella* Durrant (26).

#### THE SCAVENGER BOLLWORM, AN INSECT MISTAKEN FOR THE PINK BOLLWORM

The caterpillars of a few other species of small moths may occasionally be found in cotton bolls in the United States and have repeatedly been mistaken for the pink bollworm, causing anxiety that this dreaded pest had become established in American cotton fields.

Among such species are (*Platynota*) *Sparganothis idaeusalis* Walker and *S. rostrana* Walker, which belong to the family Tortricidae. The larvæ of these species are normally leaf-rollers on cotton and some other plants, but, especially in the fall of the year, they may enter the opened bolls, which afford convenient places for the larvæ to hibernate and pupate. These species rarely, if ever, do any actual primary damage to the bolls.

Much more common in the open cotton bolls are the small reddish caterpillars of *Pyroderces rileyi*, which, on account of their color, are likely to suggest the pink bollworm to the casual observer in the field, and which on several occasions have aroused unnecessary fears, even among entomologists.

This species is very commonly associated with cotton wherever this plant is grown, in both North America and South America, the West Indies, and the Hawaiian Islands.<sup>2</sup>

These larvæ, however, never do any independent primary injury to sound bolls, but live as scavengers in the more or less decayed dry bolls injured by other insects. The species is not confined to cotton, but feeds on dried and decayed fruits of many other plants.<sup>3</sup>

Aside from the color of the larva, there is only superficial resemblance between it and the pink bollworm; and even the color is somewhat different—much deeper and more reddish. Full grown, it is much smaller than the pink bollworm and appears more hairy because of the proportionally longer setæ; the actual number of hairs is the same in both

<sup>1</sup> Numerous experiments for the control of this species by insecticides have been recorded in literature (14, 31, 35, 38).

<sup>2</sup> A closely allied species, *Pyroderces simplex* Wlsm., is found as a scavenger in cotton in Africa.

<sup>3</sup> The species has been reported by Chittenden (48) as doing primary injury to corn in the husk, probably having been attracted to the decaying silk.



larvæ. Under a lens it is at once distinguished from the pink bollworm by the five-toothed mandibles (fig. 7) and the crotches of the abdominal prolegs, which form a complete circle (Pl. 10, C), not broken outwardly as in *P. gossypiella* (Pl. 10, K).

The following technical descriptions of *Pyroderces rileyi* will enable definite differentiation in all stages from *Pectinophora gossypiella*.

#### GENERIC DESCRIPTION

MORPH.—Labial palpi very long, recurved, sickle-shaped, reaching beyond vertex; second joint slightly thickened by smoothly appressed scales; terminal joint longer than second, smooth, acute. Antennæ simple, basal joint with strong pecten. Face, head, and thorax smooth. Forewings (fig. 5, A) narrow elongate, apex produced and pointed; 12 veins; 1b furcate at base; 2 and 3 approximate and parallel; 7 and 8

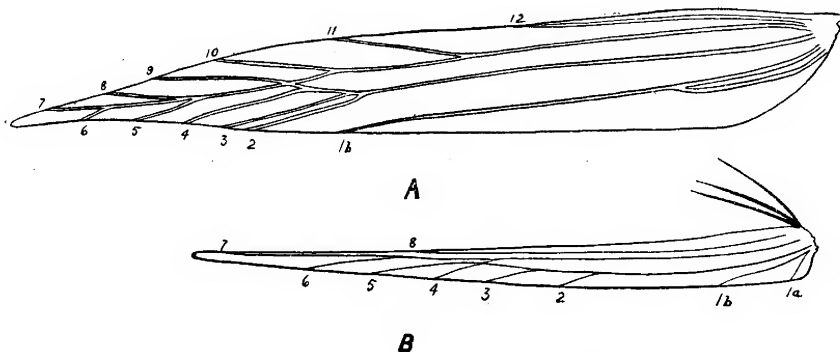


FIG. 5.—*Pyroderces rileyi*: A, Venation of forewing; B, venation of hindwing.

out of 6; 5 out of 6; 9 connate with or out of base of 6; 11 from beyond middle of cell. Hindwings (fig. 5, B) narrower than forewings, attenuated lanceolate, apex pointed; 8 veins; 6 and 7 stalked; 4 and 5 connate; 2, 3, and 4 widely separated. Posterior tibiae (Pl. 8, D) roughly haired above. Male genitalia (Pl. 8, E) with harpes and uncus developed; tegumen evenly chitinated.

LARVA.—Head (fig. 6) somewhat flattened, nearly quadrate, viewed from above, somewhat broader than long, with margin rounded and deeply incised; greatest width well behind the middle; incision of dorsal hind margin about one-third of the diameter of the head; distance between dorsal extremities of hind margin about one-half the width of the head. Front triangular, extending nearly to the incision of hind margin; adfrontal sutures straight, extending to incision of hind margin; longitudinal ridge short, not more than one-fourth as long as front. Projection of dorsal margin over ventral one-third of the diameter of the head. Triangular plates of hypostoma separated by a slightly pigmented gula, elongate. Ocelli six; i, ii, v, and vi forming a parallelogram; ii, iii, and iv in a straight line; iii and iv forward of the line between ii and v; iv and v smaller than the others. Epistoma with the usual two pairs of setæ ( $E_1$ ,  $E_2$ ) well developed. Frontal punctures ( $F_a$ ) close together, almost on a line with frontal setæ ( $F_1$ ). Distance between punctures slightly less than distance between puncture ( $F_a$ ) and setæ ( $F_1$ ); distance between frontal setæ ( $F_1$ ) and first adfrontal setæ ( $Adf_1$ ) greater than distance between adfrontal setæ  $Adf_1$  and  $Adf_2$ ; second adfrontal seta ( $Adf_2$ ) closely approximate to beginning of longitudinal ridge (LR); adfrontal puncture approximate to  $Adf_2$ .

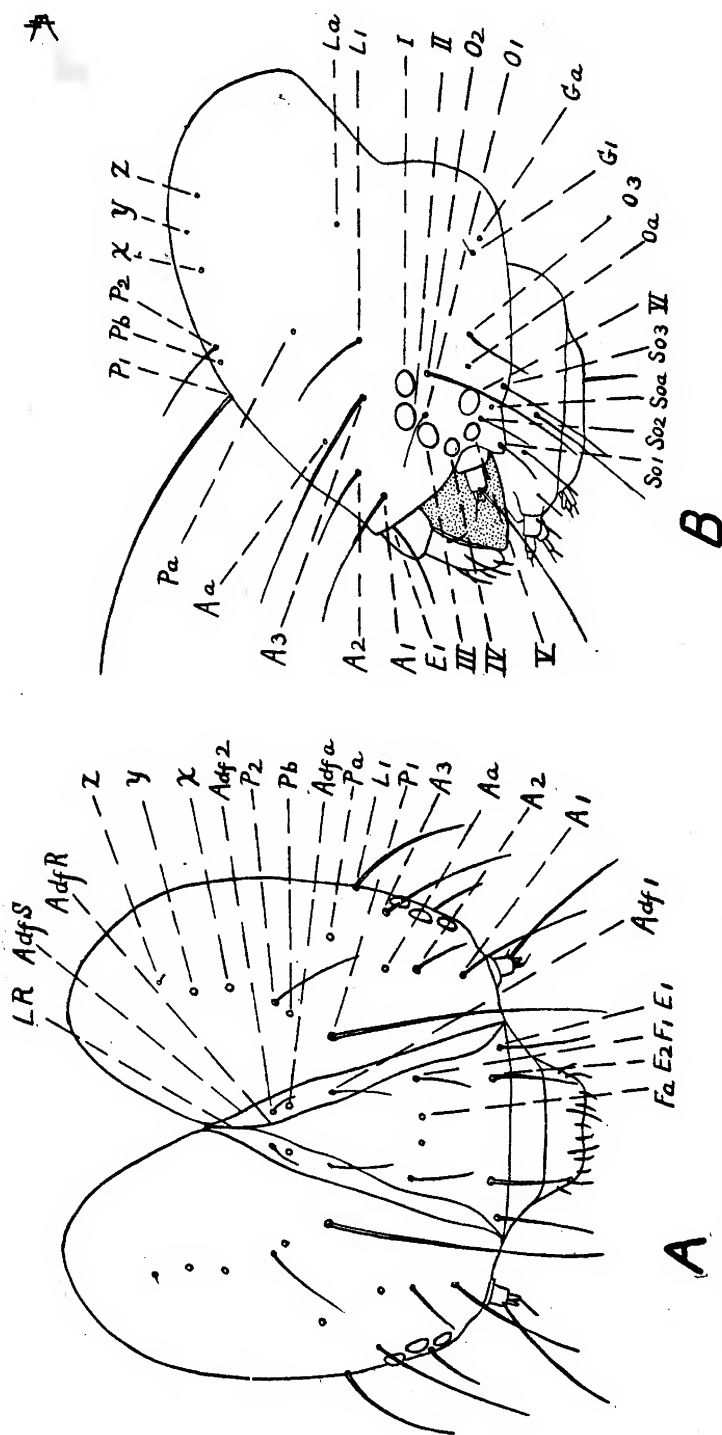


FIG. 6.—*Proderes rileyi*: A, Head capsule of larva from front. B, head capsule of larva from side. AdfR, adfrontal ridge; AdfS, adfrontal suture; LR, longitudinal ridge; E1, epistomal seta 1; E2, epistomal seta 2; F1, frontal seta; F2, frontal seta 2; A1, anterior seta 1; A2, anterior seta 2; A3, anterior seta 3; Aa, anterior puncture; Pa, posterior puncture; P1, posterior seta 1; P2, posterior seta 2; P3, posterior seta 3; P4, posterior seta 4; P5, posterior seta 5; P6, posterior seta 6; P7, posterior seta 7; P8, posterior seta 8; P9, posterior seta 9; P10, posterior seta 10; P11, posterior seta 11; P12, posterior seta 12; P13, posterior seta 13; P14, posterior seta 14; P15, posterior seta 15; P16, posterior seta 16; P17, posterior seta 17; P18, posterior seta 18; P19, posterior seta 19; P20, posterior seta 20; P21, posterior seta 21; P22, posterior seta 22; P23, posterior seta 23; P24, posterior seta 24; P25, posterior seta 25; P26, posterior seta 26; P27, posterior seta 27; P28, posterior seta 28; P29, posterior seta 29; P30, posterior seta 30; P31, posterior seta 31; P32, posterior seta 32; P33, posterior seta 33; P34, posterior seta 34; P35, posterior seta 35; P36, posterior seta 36; P37, posterior seta 37; P38, posterior seta 38; P39, posterior seta 39; P40, posterior seta 40; P41, posterior seta 41; P42, posterior seta 42; P43, posterior seta 43; P44, posterior seta 44; P45, posterior seta 45; P46, posterior seta 46; P47, posterior seta 47; P48, posterior seta 48; P49, posterior seta 49; P50, posterior seta 50; P51, posterior seta 51; P52, posterior seta 52; P53, posterior seta 53; P54, posterior seta 54; P55, posterior seta 55; P56, posterior seta 56; P57, posterior seta 57; P58, posterior seta 58; P59, posterior seta 59; P60, posterior seta 60; P61, posterior seta 61; P62, posterior seta 62; P63, posterior seta 63; P64, posterior seta 64; P65, posterior seta 65; P66, posterior seta 66; P67, posterior seta 67; P68, posterior seta 68; P69, posterior seta 69; P70, posterior seta 70; P71, posterior seta 71; P72, posterior seta 72; P73, posterior seta 73; P74, posterior seta 74; P75, posterior seta 75; P76, posterior seta 76; P77, posterior seta 77; P78, posterior seta 78; P79, posterior seta 79; P80, posterior seta 80; P81, posterior seta 81; P82, posterior seta 82; P83, posterior seta 83; P84, posterior seta 84; P85, posterior seta 85; P86, posterior seta 86; P87, posterior seta 87; P88, posterior seta 88; P89, posterior seta 89; P90, posterior seta 90; P91, posterior seta 91; P92, posterior seta 92; P93, posterior seta 93; P94, posterior seta 94; P95, posterior seta 95; P96, posterior seta 96; P97, posterior seta 97; P98, posterior seta 98; P99, posterior seta 99; P100, posterior seta 100.

Epicranium with normal number of primary setæ, 13, and punctures, 7, and with three small ultraposterior punctures (x, y, z).<sup>1</sup>

Anterior setæ ( $A_1$ ,  $A_2$ ,  $A_3$ ) in an obtuse angle;  $A_1$  and  $A_2$  closer together than  $A_2$  and  $A_3$ ; anterior puncture  $Aa$  posterior to  $A_2$  and on a line with  $A_1$  and  $A_2$ ;  $A_3$  directly lateral to  $Aa$ . Posterior setæ ( $P_1$ ,  $P_2$ ) and posterior punctures ( $Pa$ ,  $Pb$ ) near the middle of the head;  $P_1$  and  $P_2$  on a level with  $Adf_1$  and  $Adf_2$ , respectively;  $Pa$  on a line between  $L_1$  and  $P_2$ , nearest  $L_1$ ;  $Pb$  on a line between  $P_1$  and  $P_2$ , approximate to  $P_2$ . Lateral setæ ( $L_1$ ) closer to  $A_3$  than  $A_3$  is to  $A_2$ , anterior to  $P_1$ ; lateral puncture ( $La$ ) directly posterior to, and distant from the seta. Of the ocellar setæ ( $O_1$ ,  $O_2$ ,  $O_3$ )  $O_1$  is equidistant from and lateral to ocelli ii and iii;  $O_2$  closely approximate to and ventral to ocellus i;  $O_3$  posteroventral to and remote from  $O_2$ , on a line with ocellus v and vi; ocellar puncture ( $Oa$ ) between and equidistant from  $O_3$  and ocellus vi. Subocellar setæ ( $So_1$ ,  $So_2$ ,  $So_3$ ) triangularly placed, nearly equidistant; subocellar puncture ( $Soa$ ) between and equidistant from  $So_2$  and  $So_3$ . Genal seta ( $G_1$ ) and puncture ( $Ga$ ) both present; seta anterior to puncture.

Labrum (Pl. 10, A, B) with median incision acute, rather deep. The three lateral setæ ( $La_1$ ,  $La_2$ ,  $La_3$ ) close to the edge;  $La_1$  and  $La_2$  approximate,  $La_3$  remote. Median setæ ( $M_1$ ,  $M_2$ ,  $M_3$ ) in the usual Micro arrangement with  $M_2$  latera and slightly posterior to  $M_1$ ;  $M_3$  well back of anterior margin;  $M_1$  and  $M_3$  equidistant from  $M_2$ .  $M_1$  on a line with  $La_2$ ;  $M_2$  slightly anterior of  $La_1$ .

Epipharyngeal shield (ES) small, arrow shaped; epipharyngeal setæ (ET) broad plates, triangularly placed; epipharyngeal rods not discernible within the labrum proper; posterior projections short.

Mandibles (fig. 7) longer than broad; 5 teeth, 4 lower teeth long and sharply pointed; upper fifth tooth rounded; one long and one shorter seta on upper side near lower edge.

Labium and maxillæ normal.

Antennæ four-jointed, with second joint more than half the length of the entire antennæ; papillæ long, pointed; long seta more than twice the length of the antenna.

Three pairs of normal thoracic feet; four pairs of abdominal prolegs with unevenly biordinal crotches, arranged in a complete circle; anal prolegs with a transverse line of biordinal crotches.

Setal arrangement of body normal, as shown in figures C and D, Plate 11.

PUPA.—Pupa smooth, with setæ on vertex, first thoracic, and on all but the first abdominal segments. No cremaster.

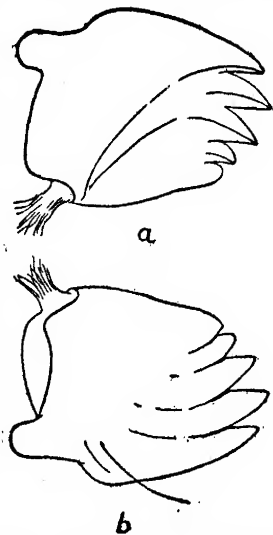


FIG. 7.—*Pyroderces rileyi*: Right mandible of larva. a, Underside; b, upper side. Greatly enlarged. (Original.)

#### SPECIFIC DESCRIPTION

MOTH (Pl. 7, B).—Labial palpi light reddish brown; second joint with two ill-defined darker brown annulations; terminal joint with three blackish annulations. Head light chestnut-brown; lower face yellowish iridescent. Antennæ whitish with sharply defined, narrow, blackish brown annulations. Forewings chestnut brown with whitish straw-colored streaks, edged by irregular black scales; an oblique whitish fascia on basal third edged on the inner side with black; an ill-defined group of black scales in the middle of the wing, edged with white; a subcostal longitudinal white streak at apical third, terminating in black scales; a similar, fainter, subdorsal streak terminating in black scales at apex of the wing; cilia yellowish gray. Hind

<sup>1</sup> Sometimes bearing minute setæ.

wings dark fuscous with paler cilia. Abdomen reddish brown. Legs (Pl. 8, *D*) light reddish, with black annulations on tarsi and tibiae.

Male genitalia (Pl. 8, *E*) weakly chitinated; harpes paddle-shaped, strongly haired; margins with row of weak curved spines; sacculus small, slightly haired; tegumen evenly chitinated; uncus moderately long, tapering to a sharp point; aedæagus long, slender, spined; inclosing the harpes ventrally is a large heart-shaped, long-haired, chitinated envelope.

Alar expanse 9 to 12 mm.

FULL-GROWN LARVA.—The full-grown larva is 7 to 8 mm. long, cylindrical, deep wine red. Head light brown, with blackish trophi. Thoracic shield broad, undivided, strongly chitinated, dark brown. Anal plate light brown. Tubercles small, whitish, bearing long, light-brown setae. Crotches of abdominal feet 20 to 24 in complete circe (Pl. 10, *C*).

PUPA.—The pupa (Pl. 12, *E, F*) is 7 to 8 mm. long, light yellowish brown, smooth, with four short setae on the vertex and six short setae on the first thoracic segment; two short, paired setae near the spiracles and four short hooked setae dorsally on fifth to ninth abdominal segments, two anterior and two posterior on each joint. Anal opening large, slitlike, surrounded by about twelve long, hooked setae; no cremaster developed; tip of abdomen bluntly rounded, armed with four long and four shorter strong, hooked setae. Fronto-clypeal suture distinct and abruptly curved upward near median line. Clypeus and pupal eyes distinctly indicated, labrum and mandibles less so; antennae close together at their tip, reaching nearly to the tips of the wings; wings reaching to posterior edge of sixth abdominal segment. Spiracles small, normal.

#### SYNONYMY OF PYRODERCES RILEYI WALSINGHAM

*Batrachedra rileyi* Walsingham (41, 45).

*Batrachedra rileyi* Howard (42, 43).

*Batrachedra rileyi* Dyar (44).

*Batrachedra rileyi* Swezey (46).

*Pyroderces rileyi* Durrant (47).

*Batrachedra rileyi* Chittenden (48).

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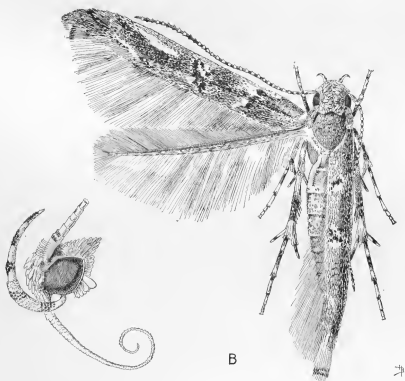
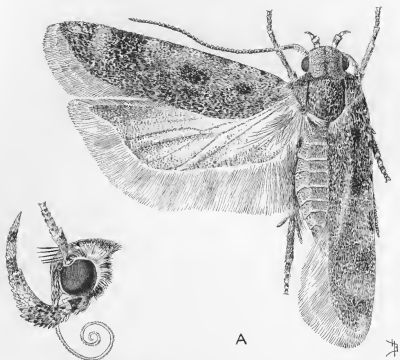
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PLATE 7

A.—*Pectinophora gossypiella*: Adult.

B.—*Pyroderces rileyi*: Adult.





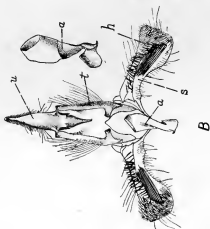
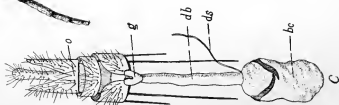
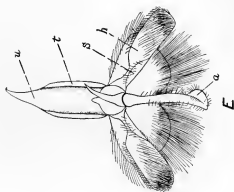


PLATE 8

A.—*Pectinophora gossypiella*: Right hindleg.

B.—*Pectinophora gossypiella*: Genitalia of male. *u*, Uncus; *t*, tegumen; *h*, harp; *a*, ædœagus; *s*, sacculus.

C.—*Pectinophora gossypiella*: Genitalia of female. *o*, Ovipositor; *g*, genital plate with genital opening; *db*, ductus bursæ; *ds*, ductus seminalis; *bc*, bursa copulatrix.

D.—*Pyroderces rileyi*: Right hindleg.

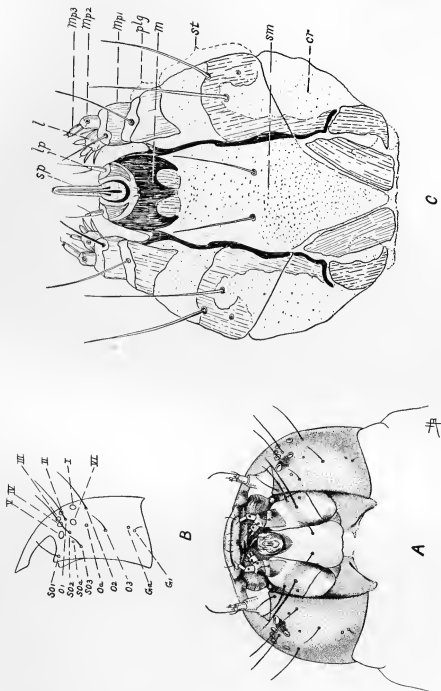
E.—*Pyroderces rileyi*: Genitalia of male. *u*, Uncus; *t*, tegumen; *s*, sacculus; *h*, harp; *a*, ædœagus.

PLATE 9

A.—*Pectinophora gossypiella*: Larval head from underside.

B.—*Pectinophora gossypiella*: Seta arrangement of epicraneum in figure A. *I*, Ocellus i; *II*, ocellus ii; *III*, ocellus iii; *IV*, ocellus iv; *V*, ocellus v; *VI*, ocellus vi; *O*<sub>1</sub>, ocellar seta 1; *O*<sub>2</sub>, ocellar seta 2; *O*<sub>3</sub>, ocellar seta 3; *Oa*, ocellar puncture; *SO*<sub>1</sub>, subocellar seta 1; *SO*<sub>2</sub>, subocellar seta 2; *SO*<sub>3</sub>, subocellar seta 3; *SOa*, subocellar puncture; *G*<sub>1</sub>, genal seta; *Ga*, genal puncture.

C.—*Pectinophora gossypiella*: Labium and maxillæ. *sp*, Spinneret; *lp*, labial palpus; *l*, lacinia and galea; *m*, mentum; *sm*, submentum; *cr*, cardo; *st*, stipes; *plg*, palpiger; *mp*<sub>1</sub>, maxillary palpus, first joint; *mp*<sub>2</sub>, maxillary palpus, second joint; *mp*<sub>3</sub>, maxillary palpus, third joint.



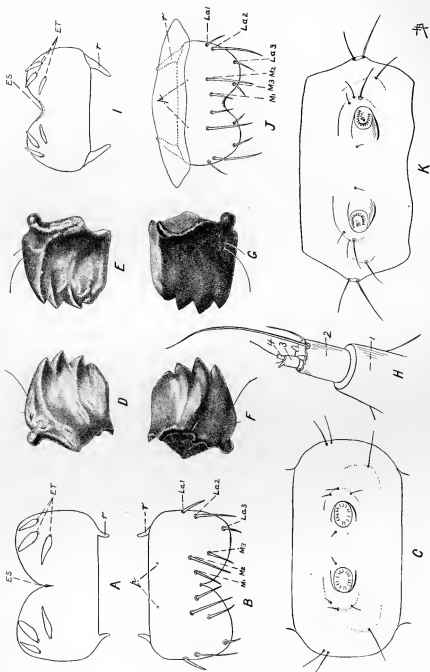




PLATE 10

A.—*Pyroderces rileyi*: Epipharynx of larva. ES, Epipharyngeal shield; ET, epipharyngeal setæ; r, epipharyngeal rod.

B.—*Pyroderces rileyi*: Labrum of larva. La<sub>1</sub>, Lateral labral seta 1; La<sub>2</sub>, lateral labral seta 2; La<sub>3</sub>, lateral labral seta 3; M<sub>1</sub>, median labral seta 1; M<sub>2</sub>, median labral seta 2; M<sub>3</sub>, median labral seta 3; p, labral punctures; r, epipharyngeal rod.

C.—*Pyroderces rileyi*: Underside of third abdominal segment of larva.

D.—*Pectinophora gossypiella*: Right mandible of larva from underside.

E.—*Pectinophora gossypiella*: Left mandible from underside.

F.—*Pectinophora gossypiella*: Right mandible from upper side.

G.—*Pectinophora gossypiella*: Left mandible from upper side.

H.—*Pectinophora gossypiella*: Left antenna of larva from underside. 1, First joint; 2, second joint; 3, third joint; 4, fourth joint.

I.—*Pectinophora gossypiella*: Epipharynx of larva. ES, Epipharyngeal shield; ET, epipharyngeal setæ; r, epipharyngeal rod.

J.—*Pectinophora gossypiella*: Labrum of larva. La<sub>1</sub>, Lateral labral seta 1; La<sub>2</sub>, lateral labral seta 2; La<sub>3</sub>, lateral labral seta 3; M<sub>1</sub>, median labral seta 1; M<sub>2</sub>, median labral seta 2; M<sub>3</sub>, median labral seta 3; p, labral punctures; r, epipharyngeal rod.

K.—*Pectinophora gossypiella*: Underside of third abdominal segment of larva.

PLATE 11

A.—*Pectinophora gossypiella*: Larva.

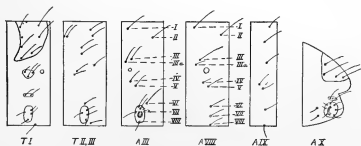
B.—*Pectinophora gossypiella*: Schematic chart of arrangement of body setæ of larva. *T I*, first thoracic segment; *T II, III*, second and third thoracic segments; *A III*, third abdominal segment; *A VIII*, eighth abdominal segment; *A IX*, ninth abdominal segment; *A X*, tenth abdominal segment.

C.—*Pyroderces rileyi*: Larva.

D.—*Pyroderces rileyi*: Schematic chart of arrangement of body setæ of larva. *T I*, first thoracic segment; *T II, III*, second and third thoracic segments; *A III*, third abdominal segment; *A VIII*, eighth abdominal segment; *A IX*, ninth abdominal segment; *A X*, tenth abdominal segment.



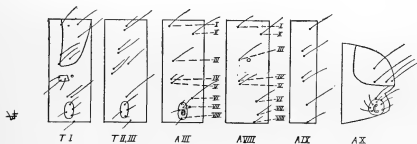
A



B



C



D

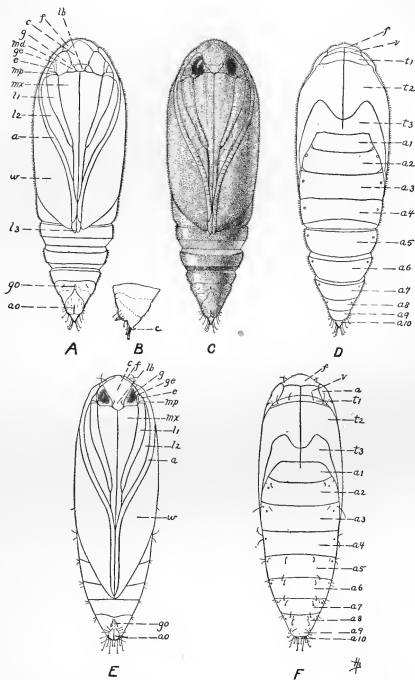


PLATE 12

A.—*Pectinophora gossypiella*: Pupa from front. *lb*, Labrum; *f*, front; *c*, clypeus; *g*, gena; *md*, mandibles; *ge*, glazed eye; *e*, eye; *mp*, maxillary palpus; *mx*, maxilla; *l*<sub>1</sub>, first thoracic leg; *l*<sub>2</sub>, second thoracic leg; *l*<sub>3</sub>, third thoracic leg; *a*, antenna; *w*, forewing; *go*, genital opening; *ao*, anal opening.

B.—*Pectinophora gossypiella*: Tip of pupa from left side. *c*, Cremaster.

C.—*Pectinophora gossypiella*: Mature pupa, with eyes of the imago visible through pupal skin.

D.—*Pectinophora gossypiella*: Pupa from back. *f*, Front; *v*, vertex; *t*<sub>1</sub>, first thoracic segment; *t*<sub>2</sub>, second thoracic segment; *t*<sub>3</sub>, third thoracic segment; *a*<sub>1</sub>, first abdominal segment; *a*<sub>2</sub>, second abdominal segment; *a*<sub>3</sub>, third abdominal segment; *a*<sub>4</sub>, fourth abdominal segment; *a*<sub>5</sub>, fifth abdominal segment; *a*<sub>6</sub>, sixth abdominal segment; *a*<sub>7</sub>, seventh abdominal segment; *a*<sub>8</sub>, eighth abdominal segment; *a*<sub>9</sub>, ninth abdominal segment; *a*<sub>10</sub>, tenth abdominal segment.

E.—*Pyroderces rileyi*: Pupa from front. *c*, Clypeus; *f*, front; *lb*, labrum; *g*, gena; *gl*, glazed eye; *e*, eye; *mp*, maxillary palpus; *mx*, maxilla; *l*<sub>1</sub>, first thoracic leg; *l*<sub>2</sub>, second thoracic leg; *a*, antenna; *w*, forewing; *go*, genital opening; *ao*, anal opening.

F.—*Pyroderces rileyi*: Pupa from back. *f*, Front; *v*, vertex; *a*, antenna; *t*<sub>1</sub>, first thoracic segment; *t*<sub>2</sub>, second thoracic segment; *t*<sub>3</sub>, third thoracic segment; *a*<sub>1</sub>, first abdominal segment; *a*<sub>2</sub>, second abdominal segment; *a*<sub>3</sub>, third abdominal segment; *a*<sub>4</sub>, fourth abdominal segment; *a*<sub>5</sub>, fifth abdominal segment; *a*<sub>6</sub>, sixth abdominal segment; *a*<sub>7</sub>, seventh abdominal segment; *a*<sub>8</sub>, eighth abdominal segment; *a*<sub>9</sub>, ninth abdominal segment; *a*<sub>10</sub>, tenth abdominal segment.



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## TOXICITY OF VARIOUS BENZENE DERIVATIVES TO INSECTS

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### INTRODUCTION

The author in a previous paper (5)<sup>1</sup> pointed out the possibility of fumigating animals with nitrobenzene<sup>2</sup> to destroy their external parasites. In that work and in later experiments with nitrobenzene as many as 500 animals (cattle, sheep, hogs, chickens, dogs, cats, rats, and guinea pigs) have been fumigated, with only two cases of possible poisoning. One case was the fumigation of five chickens, which, through a misunderstanding of an assistant, were fumigated for 13 hours instead of 8, with the result that the chickens later died from paralysis of the central nervous system. The other instance was that of a young cat, which after one hour showed signs of uneasiness and was removed from the fumigation cage. As no symptoms of poisoning resulted in this case the cat may have been reacting to a strange environment rather than to the action of the poison.

Nevertheless, in view of the extreme toxicity of nitrobenzene as recorded in different works on toxicology, it was felt that it might be too poisonous for general use by inexperienced persons. For this reason a study of a series of benzene derivatives was undertaken with a view to determining their toxicity to insects; and from the result of this study it was hoped that one or more compounds might be found which would be quite toxic to insects while nontoxic to higher animals or plants. A study of the toxicity of the vapor of 28 benzene derivatives has been completed. A knowledge of the toxicity of the vapors of these compounds is valuable not alone for fumigation purposes but also as an

<sup>1</sup> Reference is made by number to "Literature cited," p. 380.

<sup>2</sup> In nomenclature the usage of American Chemical Society is followed.

index of their worth as contact sprays, since Shafer (6) and, more recently, McIndoo (4) have shown that most contact sprays kill by the action of their vapor rather than by the plugging of the spiracles.

#### COMPOUNDS USED IN THE EXPERIMENTS

From the hydrocarbon benzene  $C_6H_6$  a great many compounds may be derived by replacement of one or more of the hydrogen atoms by certain other elements or groups of elements. These compounds are designated "mono," "di," "tri," etc., derivatives, depending on the number of hydrogens which are substituted. The following mono-substitution compounds have been tested in this study:

Benzonitrile, $C_6H_5CN$	Anilin, $C_6H_5NH_2$
Chlorbenzene, $C_6H_5Cl$	Benzaldehyde, $C_6H_5CHO$
Brombenzene, $C_6H_5Br$	Nitrobenzene, $C_6H_5NO_2$
Iodobenzene, $C_6H_5I$	Toluene, $C_6H_5CH_3$
Phenol, $C_6H_5OH$	

The following di-substitution products were employed:

Xylene, $C_6H_4(CH_3)_2$ (mixture of the three possible isomers)	Ortho-chlorphenol, $C_6H_4OH Cl$
Para-dichlorbenzene, $C_6H_4Cl_2$	Ortho-nitrophenol, $C_6H_4OH NO_2$
Para-dibrombenzene, $C_6H_4Br_2$	Salicylic aldehyde, $C_6H_4O H CHO$

Besides these di-substitution compounds, several other derivatives were used, which may be considered di-substitution compounds of benzene or mono-substitutions of toluene. They were ortho- and para-bromtoluene ( $C_6H_4CH_3Br$ ), ortho-, meta-, and para-cresol ( $C_6H_4CH_3OH$ ), and ortho-nitrotoluene ( $C_6H_4CH_3NO_2$ ). Inasmuch as different compounds are obtained by substitution in the methyl group of toluene rather than in the benzene ring of toluene, two such compounds were tested: Benzyl alcohol ( $C_6H_5CH_2OH$ ) and benzoyl chlorid ( $C_6H_5CO Cl$ ). Two derivatives of xylene were tried: Bromxylene ( $C_6H_3(CH_3)_2Br$ ) and nitroxylene ( $C_6H_3(CH_3)_2NO_2$ ).

The xylene used in the experiments was a mixture of ortho-, meta-, and para-xylene; hence, the bromxylene and nitroxylene were also mixed compounds.

In this series is shown a wide range of compounds very different in chemical composition. A few others were tested but not included, owing to their slight volatility.

#### METHODS OF EXPERIMENTATION

One-liter Florence flasks of pyrex glass, closed with rubber stoppers, were used as fumigation chambers. As rubber was found to absorb the vapor of the chemicals, the stopper was coated with lead foil. Measured quantities of the compound to be tested were placed on a piece of filter paper cut just as small as possible, the paper was suspended from the

stopper inside of the flask, and the compound was allowed to evaporate. After several different insects were used in preliminary tests, the house fly (*Musca domestica* L.) was selected as being typical and easy to breed in large numbers. The flies were bred in the insectary and kept under natural conditions, thus avoiding irregular results due to the different ages and physical conditions of the wild flies. Five flies were put into each flask, the chemical introduced, and the flask tightly stoppered. When all the flies in the flask were apparently dead, they were removed to a vial and given 24 hours to revive. If none revived, the time during which the flies were exposed to the vapor was recorded. But, if the flies revived, the experiment was repeated. The average of 50 tests for a certain quantity of any chemical was found to be practically the same as the average of 5 tests; hence, in each case 5 tests were conducted. Controls showed that flies could live in a closed flask for 20 or more hours.

Since similar weights of the different chemicals do not contain the same number of molecules, and their toxicity could not, therefore, be accurately compared, it was decided to determine the toxicity in minutes for similar fractions of a gram-molecule of each chemical. Different quantities of each chemical were tested and curves plotted. As the quantity increased, it was found that each chemical had a point beyond which an increase would not give a reduction in the time required to kill. This is the point at which the air is saturated with the vapor, and differs for each chemical. As the quantity is decreased, a point is reached where the vapor is not of sufficient strength to kill. The plotted curves lie between these two points.

After the curves were plotted, it was found to be impossible to compare similar fractions of a gram-molecule; hence, the different fractions of a gram-molecule necessary to kill in a fixed time of 400 minutes were determined. A long period of time was selected as a more nearly correct index of toxicity. The fraction of the gram-molecule was determined by dividing the amount of the chemical necessary to kill in 400 minutes by the molecular weight of the substance. The sums given in the charts are the millionths of a gram-molecule necessary to kill five house flies in a 1-liter flask at a temperature of 70° F.

The liquid benzene compounds were measured by volume in blood-counting pipettes, and the weight of this volume was determined from the weight of 1 c. c. of the chemical. Weighed quantities of the solid benzene derivatives were dissolved in a known volume of benzene. A certain volume of this solution would contain a definite quantity of the benzene derivative. The measured volume was placed on the paper and blown for a moment to evaporate the solvent. The rapid evaporation of the solvent resulted in a lowering of the temperature, thus preventing appreciable evaporation of the compound to be tested.



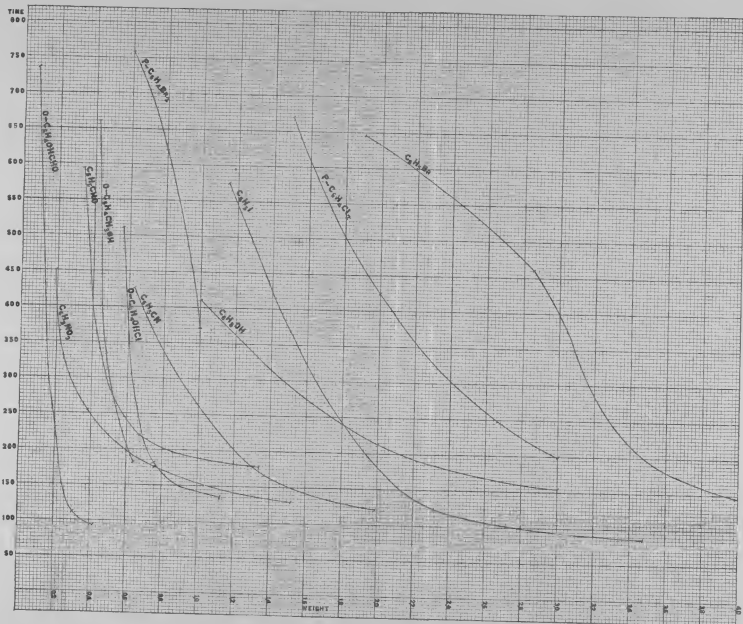


FIG. 1.—Curves showing periods of time required to kill with different quantities of the different benzene derivatives.



TABLE I.—Quantity of a chemical and time required to kill five house flies in a 1-liter flask at 70° F.

Chemical.	Quantity of chemical required.	Time required (average of five tests).	Chemical.	Quantity of chemical required.	Time required (average of five tests).
	Gm.	Minutes.		Gm.	Minutes.
Carbon bisulphid.....	0.02179	400	Salicylic aldehyde.....	.00044	93
Benzene.....	.02028	185	Do.....	.00029	110
Do.....	.01521	349	Do.....	.00014	349
Do.....	.01015	427	Do.....	.00007	735
Toluene.....	.01548	258	Ortho-nitrophenol.....	.001	241
Do.....	.01289	498	Do.....	.0007	409
Do.....	.01032	600	Do.....	.0005	512
Chlorbenzene.....	.01265	85	Ortho-bromtoluene.....	.00265	159
Do.....	.00632	215	Do.....	.00177	341
Do.....	.00413	506	Do.....	.00132	610
Brombenzene.....	.00475	133	Para-bromtoluene.....	.001	318
Do.....	.0038	160	Do.....	.0005	350
Do.....	.0028	461	Do.....	.00025	351
Do.....	.0019	646	Do.....	.0001	544
Phenol.....	.003	155	Ortho-cresol.....	.00064	182
Do.....	.002	213	Do.....	.00048	333
Do.....	.001	411	Do.....	.00042	661
Iodobenzene.....	.00347	87	Meta-cresol.....	.00196	223
Do.....	.00231	123	Do.....	.00130	304
Do.....	.00115	576	Do.....	.00065	479
Benzonitrile.....	.00190	122	Para-cresol.....	.00193	249
Do.....	.00137	166	Do.....	.00129	319
Do.....	.00063	427	Do.....	.00064	360
Anilin.....	.00128	137	Do.....	.00032	435
Do.....	.00064	204	Benzoyl chlorid.....	.00257	130
Do.....	.00048	511	Do.....	.00164	273
Benzaldehyde.....	.00134	178	Do.....	.00082	486
Do.....	.00067	219	Ortho-nitrotoluene.....	.00058	281
Do.....	.00033	595	Do.....	.00036	293
Nitrobenzene.....	.00152	130	Do.....	.00029	450
Do.....	.00076	176	Bromxylene (B. P.		
Do.....	.00038	254	190°-250° C.).....	.002	201
Do.....	.00019	451	Do.....	.001	292
Xylene.....	.01005	95	Do.....	.0005	487
Do.....	.00754	305	Bromxylene (B. P.		
Do.....	.00502	911	220°-250° C.).....	.00087	269
Para-dichlorbenzene ..	.003	200	Do.....	.00043	356
Do.....	.002	424	Do.....	.00022	532
Do.....	.001	670	Nitroxylene.....	.00028	368
Para-dibrombenzene ..	.001	371	Do.....	.00014	581
Do.....	.0008	621	Furfural.....	.00297	155
Do.....	.0006	759	Do.....	.00223	273
Ortho-chlorphenol.....	.00112	131	Do.....	.00198	437
Do.....	.00075	181			
Do.....	.00056	512			

TABLE II.—*Quantity of a chemical necessary to kill five house flies in a 1-liter flask in an arbitrary time of 400 minutes*

Chemical.	Quantity of chemical required (in millionths of a gram-molecule).	Chemical.	Quantity of chemical required (in millionths of a gram-molecule).
Carbon bisulphid.....	286.3	Salicylic aldehyde.....	1.1
Benzene.....	142.3	Ortho-nitrophenol.....	5.6
Toluene.....	147.5	Ortho-bromtoluene.....	9.4
Chlorbenzene.....	42.4	Para-bromtoluene.....	1.2
Brombenzene.....	19.2	Ortho-cresol.....	4.2
Phenol.....	10.8	Meta-cresol.....	7.9
Iodobenzene.....	6.6	Para-cresol.....	3.9
Benzonitrile.....	6.4	Benzyl alcohol.....	5.3
Anilin.....	5.3	Benzoyl chlorid.....	7.8
Benzaldehyde.....	3.7	Ortho-nitrotoluene.....	2.1
Nitrobenzene.....	1.8	Bromxylene (B. P. 190°-210° C.)	3.5
Xylene.....	6.4	Bromxylene (B. P. 220°-250° C.)	1.9
Para-dichlorbenzene.....	14.0	Nitroxylene.....	1.7
Para-dibrombenzene.....	4.1	Furfural.....	20.8
Ortho-chlorphenol.....	4.6		

## DISCUSSION OF RESULTS

## TOXICITY AND CHEMICAL COMPOSITION

By a glance at figure 2 it is noticed that all the benzene compounds used are more toxic than carbon bisulphid. The introduction of a methyl group into the benzene ring decreases its toxicity. This result agrees with the findings for higher animals of Winternitz and Hirschfelder (7) and further studies of Kline and Winternitz (3). The introduction of a halogen increases the toxicity similar to the results of Bechhold and Ehrlich (1), who found the introduction of a halogen increased the disinfection properties of phenol. This fact is true for insects whether the halogen is introduced in benzene, toluene, xylene, or phenol.

One might expect that, as toluene is less toxic than benzene, the halogen derivative of toluene would be less toxic than the similar derivative of benzene; but such does not seem to be the case. The iodine derivatives are more toxic than the corresponding bromine compounds, while both are more toxic than the corresponding chlorine derivatives. The disubstitution compounds of the halogens are more toxic than the monosubstitutions. The introduction of the cyanogen group does not increase the toxicity as much as might be supposed. The aldehyde group greatly increases the toxicity; in fact, salicylic aldehyde is the most toxic of all the compounds used in the experiments. From this result it would be expected that furfural would be much more poisonous than the results show it to be. Substitutions in the methyl group of toluene are

less toxic than in the benzene ring. Para configurations seem to be more toxic than ortho configurations, while the only meta derivative tried was less toxic than either. Although certain relationships exist between chemical composition and toxicity, they are not as striking or as constant as might be expected.

#### BOILING POINTS AND TOXICITY

In working over the results, the author noticed a relationship between the boiling point of the chemical and its toxicity. As many of the compounds bore no guaranty of purity, the boiling points of several were determined and a curve plotted, showing the chemicals in order from the lowest boiling point to the highest. In comparison, a curve of toxicity of these compounds was plotted, as shown in figure 3. The curves show strikingly that the higher the boiling point the more toxic is the chemical. Exceptions are to be noted, which may be due to the rôle played by chemical composition in either raising or lowering the toxicity; but, in general, the curve is an increase of toxicity with an increase in the boiling point. Benzaldehyde shows a break in the curve, possibly owing to a specific action of the aldehyde. The low boiling point of furfural ( $96^{\circ}$  C.) may account for its toxicity being less than would be expected from its chemical composition. Carbon bisulphid, having the lowest boiling point ( $47^{\circ}$  C.), lower than any of the benzene derivatives tested, is likewise the least toxic of all the compounds.

The explanation of the relationship of boiling point to toxicity has not been ascertained. Whether the introduction of a certain element or group causes an increase in toxicity incidental to an increase in the boiling point or whether it is the relationship of boiling point to vapor pressure and volatility is not known.

#### BOILING POINT AND LIPOID SOLUBILITY

Another interesting observation is the relationship between boiling point and lipid solubility. To test the lipid solubility of the compounds, cephalin was extracted from the brain of an ox by Hirschfelder's method (2).

Ox brain was covered with three volumes of alcohol, shaken up two or three times, and the excess of alcohol then poured off and squeezed out gently through linen, care being taken to avoid great force in wringing out the alcohol, as this tends to break up the brain tissue into very finely divided particles which pass through the filter. The residue is then covered with three volumes of ether, shaken vigorously, and filtered first through cotton and then through filter paper. The clear filtrate thus obtained is evaporated to dryness over a water bath and a yellow residue remains.

The cephalin so prepared was placed in capsule heads of 0.08 c. c. capacity and introduced into 1 c. c. of the chemical to be tested. It was found that benzene boiling at  $78.5^{\circ}$  C., toluene at  $107.5^{\circ}$  C., and xylene at  $130^{\circ}$  C. dissolved several capsules of cephalin until it finally

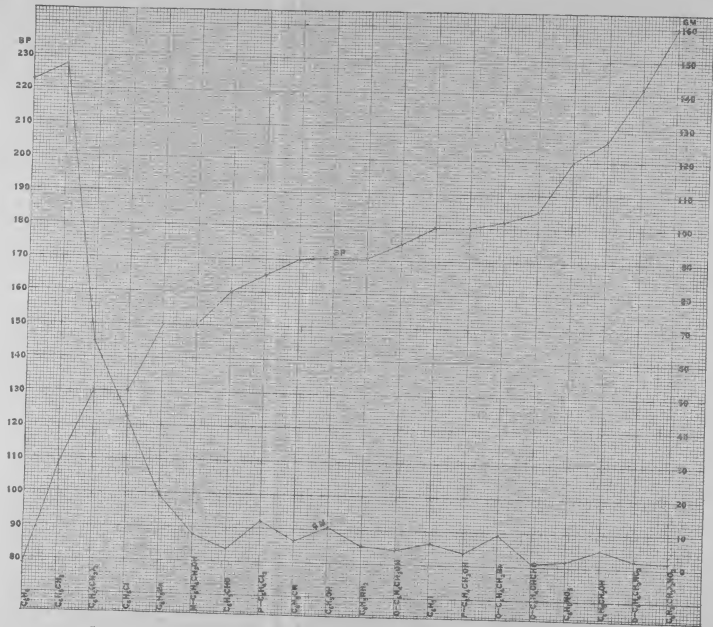


FIG. 2.—Curves showing the relationship between boiling point (BP) of the compound and its toxicity expressed in millionths of a gram-molecule (GM).  
94351°—17. (To face page 377.)

became thick and pastelike. By placing cephalin under a small bell jar, the air of which remained saturated with benzene, it absorbed benzene from the air until finally a liquid mass was produced. The same was true for toluene and xylene. This shows that cephalin and either benzene, toluene, or xylene are miscible in all proportions. On the other hand, brombenzene, with a boiling point of  $150^{\circ}\text{C}$ ., dissolved but one capsule in 1 c. c. Benzaldehyde ( $165^{\circ}\text{C}$ .) slowly penetrated the cephalin, but dissolved but little of it; while anilin ( $170.5^{\circ}\text{C}$ .) salicylic aldehyde ( $185^{\circ}\text{C}$ .), nitrobenzene ( $200^{\circ}\text{C}$ .), and nitroxylene ( $240^{\circ}\text{C}$ .) did not penetrate the cephalin and dissolved but very slight traces of it. Five c. c. of nitrobenzene, evaporated to dryness, left a very slight greasy mark on the evaporating dish. An effort was made to extract cephalin from the brain tissue with nitrobenzene without success. Lanolin also is practically insoluble in nitrobenzene. One c. c. of benzene containing 0.16 c. c. of cephalin was poured into 10 c. c. of nitrobenzene and the mixture blown with an electric fan until the benzene was evaporated, resulting in the cephalin's being thrown out of solution. From these results it appears that compounds with high boiling points are poor lipoid solvents, but are the most toxic to insects. These experiments would indicate that an increase in lipoid solubility as determined by the above method causes a decrease in toxicity in the chemicals used. Further work is now in progress to determine whether a similar relationship exists between the boiling point, lipoid solubility, and toxicity of a wider range of chemicals from the alipathic series and the terpenes.

#### TOXICITY OF BENZENE DERIVATIVES TO OTHER INSECTS

The toxicity of the benzene derivatives was found to be similar for other insects, and although this work has not been completed, one point may be noted. A comparison of the bluebottle fly (*Lucilia sericata* Mg.) with the house fly (*Musca domestica* L.) shows that house flies die more quickly from compounds with a low boiling point than bluebottle flies, while compounds with a high boiling point are more toxic to the bluebottle flies than to the house fly. Similarly, the cockroach (*Blatella germanica* Linn.) succumbs less readily than the potato beetle (*Leptinotarsa decemlineata* Say) to low boiling compounds and more readily to high boiling compounds. This relationship may be due to morphological differences in the insects, possibly the diameter of the spiracles or trachea.

#### CONCLUSIONS

Although no effort has yet been made to apply the results, certain possibilities are apparent. Even if the compounds with low boiling points are less toxic than those with high boiling points, inasmuch as more of such compounds may be evaporated before saturation is reached, better results may be obtained. This is shown in figure 4, which gives the maximum amount (in pounds) that will evaporate in 1,000 cubic feet of



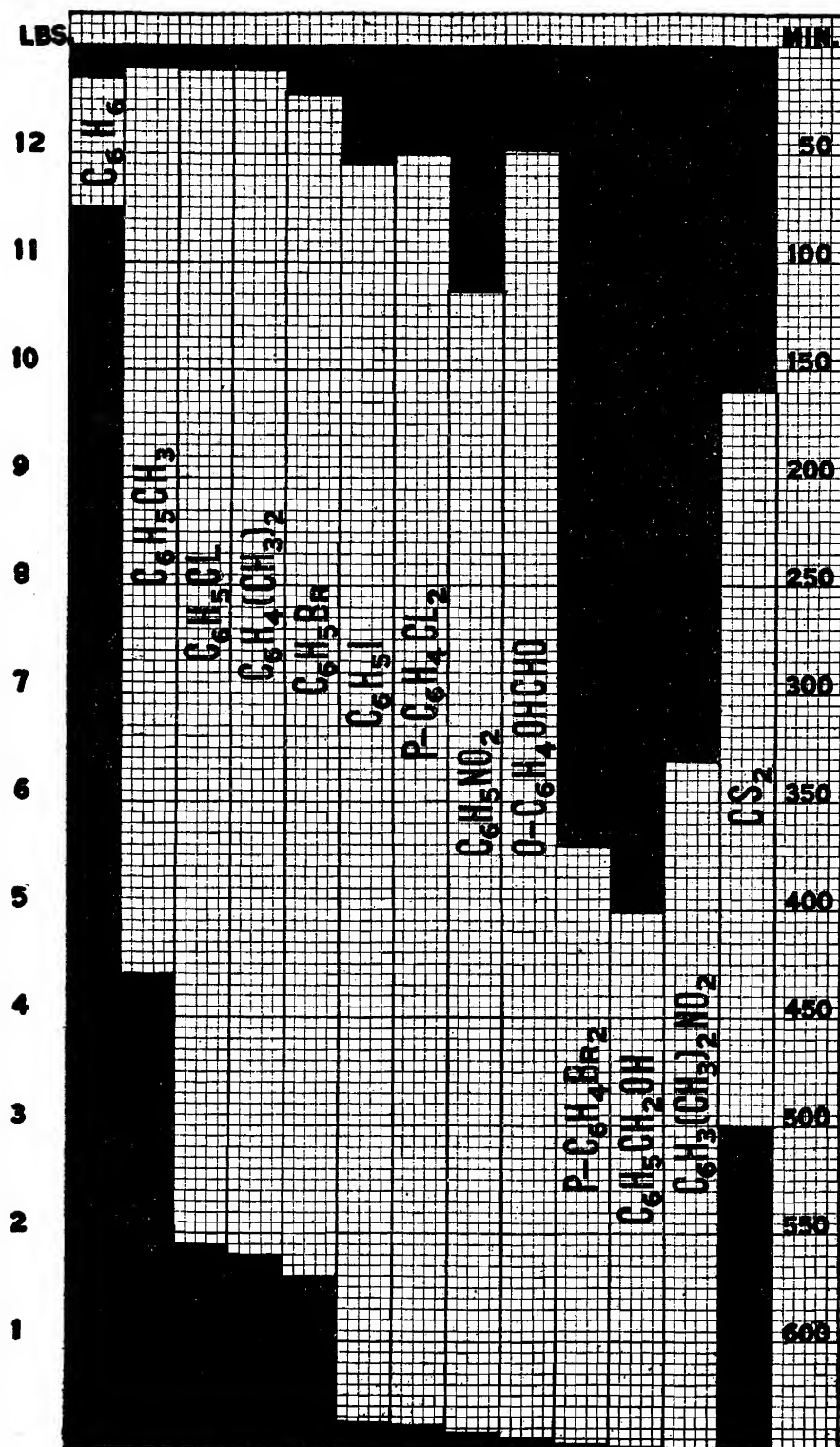


FIG. 4.—Graph showing the quantity of the benzene derivatives necessary to saturate 1,000 cubic feet of space at 70° F. and the time required by such quantity to kill house flies. Carbon bisulphid at the standard rate is given for comparison.

space at 70° F., and the time required for such quantity to kill house flies. Carbon bisulphid at the rate of 3 pounds to 1,000 cubic feet is compared with the benzene derivatives. As a low-boiling compound will penetrate grain better than a high-boiling compound, the possibilities of xylene, chlorbenzene, and brombenzene are at once apparent. Tests of the value of these compounds in the fumigation of grain have not been made. Inasmuch as the vapor of many of the benzene compounds is explosive when mixed with air, one must observe certain precautions, although in general they are far less explosive than carbon bisulphid.

For the fumigation of animals a compound with a high boiling point is needed in order that relatively little of the material shall be in the air to be taken in by the animal or to irritate the eyes or nose. In this respect salicylic aldehyde is probably the best. The cost of this chemical is prohibitive for general fumigation; but, inasmuch as higher animals readily oxidize it to salicylic acid, which is very slightly poisonous, this compound might be used for the internal fumigation of horses to destroy bots as carbon bisulphid is now used. As previously stated, it has been decided to try out a large series of chemicals before selecting the best compounds for tests as to their practicable possibilities.

#### SUMMARY

Data are presented showing the toxicity of certain organic compounds, mainly from the aromatic series, to insects, particularly the house fly, and certain general relationships are indicated.

(1) All the benzene derivatives tested proved to be more toxic to insects, molecule for molecule, than carbon bisulphid.

(2) Physical characters, such as boiling point and vapor pressure, have more influence on the toxicity than chemical composition.

(3) Up to 250° C. the higher the boiling point the more toxic the compound to insects. Beyond 250° C. the compound is usually so slightly volatile that not enough of the chemical will evaporate to be effective.

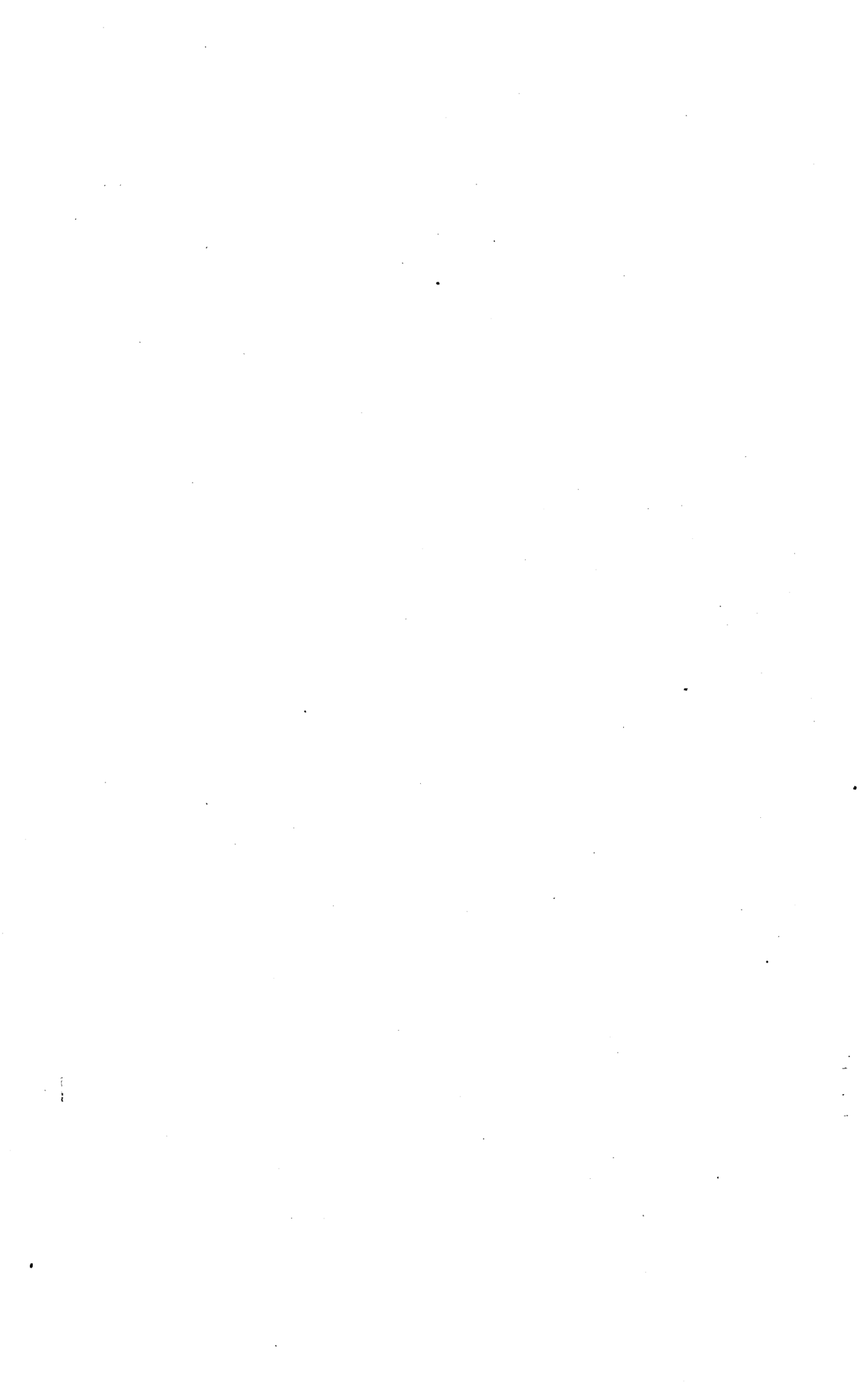
(4) Lipoids are very soluble in compounds with low boiling points and but slightly soluble in compounds with high boiling points.

(5) Compounds with low boiling points, although less toxic, owing to their great volatility, may give better results than compounds with high boiling points, particularly in the fumigation of grain.

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# HYBRIDS OF *ZEa RAMOSA* AND *ZEa TUNICATA*

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## INTRODUCTION

Data regarding the domestication of maize are still extremely meager. Yet a knowledge of this important agricultural step would be of such importance in tracing the early history of man and the beginnings of civilization that any investigations promising to throw light on the subject need no further justification.

The present study deals with the behavior of a hybrid between the two most striking variations or mutations from normal maize. Both have been considered as distinct species, *Zea tunicata* and *Zea ramosa*. Though usually referred to as agricultural species, they seem to deserve a place with the so-called species of *Oenothera*, which have originated through mutation.

## DESCRIPTION OF PARENTS

*Zea tunicata*, or "pod corn," is a rather well-known variation of ordinary maize (Pl. 13, 14, 15). The most striking characteristic is that the glumes of the female inflorescence, or ear, are developed so that each seed is entirely inclosed. Associated with this character is a less conspicuous lengthening of the glumes of the staminate inflorescence that results in a thickening of the tassel (Pl. 13, B).

The origin of *Z. tunicata* is not known, but its occurrence in widely separated and isolated regions would indicate that it has originated independently more than once, presumably as a mutation from ordinary maize. So far as known, it has never appeared in pedigreed cultures, but there is at least one instance where it is reported to have appeared in a carefully bred commercial variety (Sconce, 1912).<sup>1</sup>

*Z. tunicata* is reported from Paraguay, Brazil, Argentina, Belgian Congo, and many places in the United States. It was known to many tribes of American Indians. According to Parker (1910), both the Senecas and Mohawks had special names for tunicate maize, that in Seneca being translated as "original corn."

Thus far we have found no definite reference to tunicate maize in Mexico, the reference given by Sturtevant (1894) being obviously a misidentification. Neither has it been reported from Peru, and in a most extensive vocabulary of the Quichua terms relating to maize obtained in Peru by Mr. O. F. Cook, of the Bureau of Plant Industry, who made

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<sup>1</sup> Bibliographic citations in parentheses refer to "Literature cited," p. 395.



special inquiry for this type of maize among well-informed people familiar with Indian agriculture, there is no mention of tunicate maize.

In hybrids with nontunicate varieties the tunicate character behaves as a dominant, but in our experiments we have never been able to secure a homozygous tunicate strain. Progenies resulting from the selfing of tunicate plants have, with us, always shown segregation into approximately three tunicate plants to one normal.<sup>1</sup>

The tunicate plants in self-pollinated progenies are separable into two classes, one producing typical tunicate ears and thickened tassels like the parent plant (Pl. 13, B; 14, B), the other with greatly enlarged tassels containing both staminate and pistillate flowers, and with the ear either aborted or bearing greatly enlarged and usually sterile spikelets (Pl. 13, A; 14, B; 15). This last class represents approximately one-third of the tunicate plants. Although these plants produce what appears to be normal pollen in the terminal inflorescence, the long glumes never open and the pollen is not shed; and we have not been successful in securing selfed seed of this form.

The ratios in which the different classes occur would indicate that the class with the bisexual terminal inflorescence is the homozygous form and that the ordinary tunicate plants represent the heterozygous form, a cross between the form with the bisexual inflorescence and the normal nontunicate maize. If the ordinary tunicate type can occur in a homozygous form, we should expect one in four of the plants grown from a self-pollinated tunicate plant to be homozygous, and the progeny of such homozygous plants should be all podded, whether cross or self-pollinated. This has not been the case in our experiments. If only a few progenies were grown, the failure to secure an all-tunicate progeny might, of course, be ascribed to the accidental selection of heterozygous instead of homozygous parents.

In the course of our experiments the progenies of 43 different tunicate plants have been grown and in all of these progenies, except one, nontunicate plants appeared. The one exception produced only eight plants. And, since only two or three normal plants were expected, it is not surprising that none appeared. Of the remaining 42 parent plants, 14 might have been expected to prove homozygous. That none proved to be homozygous can hardly be accidental, since the chances against it are over 400,000 to 1. It is therefore concluded that, in the material that has come under our observation, the ordinary type of tunicate plants represents a case of imperfect dominance, and that it is unfixable,

<sup>1</sup> A comparison of the ratios of tunicate to nontunicate plants shows the following:

	Tunicate.	Non-tunicate.
Self and tunicate × tunicate.....	288	94
Expected.....	286.5 ± 5.7	95.5
Tunicate × nontunicate.....	99	117
Expected.....	108 ± 5.0	108

like the Andalusian fowls. Our experiments have contained tunicate strains from three distinct sources; but, since other workers report the existence of pure-tunicate strains, it may be that still other stocks behave differently.

The distinction between full tunicate and half tunicate has not always been made in our pedigrees, but the records show 17 progenies where the number of full-tunicate plants is recorded. The total number of plants in these 17 progenies is 187, of which 46 were classed as full tunicate. If, as suggested, the full-tunicate plants are the homozygous form, the expected for the number of individuals involved would be 62, a deviation of 16, or four times the probable error, a rather large deviation to be ascribed to chance, but not sufficiently aberrant to offset the failure to secure homozygous individuals among the half-tunicate plants. The distinction between full and half tunicate is not always easy to make, and it would appear from the ratios that we have been referring some of the less pronounced examples of the full tunicate to the half-tunicate class. By concentrating selection on this group of plants, more or less intermediate between full and half tunicate, it may be possible to secure a homozygous strain. But in the stocks with which we have been experimenting, individuals of the type shown in Plate 13, A, or that shown by East and Hayes (1911) would not serve as examples of pure-tunicate maize.

The class with bisexual terminal inflorescence, which is here assumed to be homozygous, will be referred to as "full tunicate" and the ordinary tunicate type, which we look upon as heterozygous, will be termed "half tunicate." The term "tunicate," or podded, will be used as a general term including both of the above classes.

*Z. ramosa*, or branched maize, is a variation from ordinary maize discovered by Dr. W. B. Gernert (1912) at the Illinois Agricultural Experiment Station. The original ear was found in 1909 in a field of Leaming corn.

*Z. ramosa* differs from the normal maize in having the pistillate inflorescence, or ear, which is normally simple, replaced by a compound inflorescence branched like the tassel (Pl. 14, A, c). There is also a less striking but equally significant change in the branching of the tassel (Pl. 16). In normal maize the terminal inflorescence bears a number of branches at its base. Above the uppermost branch the axis is continued into what is termed the "central spike," where the pairs of spikelets are borne directly on the main axis of the inflorescence. Thus, in passing from the base to the tip of the tassel, there is an abrupt transition from the uppermost branch to simple pairs of spikelets. In the *Z. ramosa* tassel the branches are much more numerous and gradually decrease in size from the base upward, the transition from branches to pairs of spikelets being imperceptibly gradual.

Unlike *Z. tunicata*, *Z. ramosa* is a recessive variation. The dominance of normal maize over this variation seems complete. We have never been able in any way to distinguish between plants heterozygous for the *ramosa* character and normal maize. So far as observed, the character behaves as a simple Mendelian unit.

Krafft (1870) described and figured a branch pistillate inflorescence that appears to have been of the same character as *Z. ramosa*. The reference is of interest as indicating that *Z. ramosa*, as well as *Z. tunicata*, may be looked upon as having originated more than once.

#### NATURE OF THE VARIATIONS

In normal maize one of the characters differentiating the male and female inflorescence is that the glumes are greatly reduced in the pistillate inflorescence, so that the kernels are naked on the cob. In *Z. tunicata* this differentiation is lost, and the kernels are inclosed in glumes as long as those of the staminate inflorescence.

Another specialized character of normal varieties of maize is the suppression of the branches in the pistillate inflorescence, or ear. In *Z. ramosa* this specialization is lost, and the ear is as completely branched as the tassel. A further loss of specialization in *Z. ramosa* is that the differentiation between branches and pairs of spikelets is lost, the one grading into the other in both ear and tassel.

In considering these losses of specialization it is desirable to keep in mind the fact that in *Z. tunicata*, as in ordinary maize, the ear is a pistillate homologue of the central spike of the tassel, while in *Z. ramosa* the ear is a pistillate replica of the entire tassel. The loss of specialization in *Z. tunicata* affects the characters of the floral organs and spikelets, while in *Z. ramosa* the general form of the pistillate inflorescence is changed to conform to that of the tassel.

Both *Z. ramosa* and *Z. tunicata* are variations from normal maize toward the general type of grasses, and as such may be looked upon as reversions, since both cases involve a loss of a specialization that distinguishes maize from practically all other grasses.

Recent investigations have shown that many reversions may be explained as recombinations, but neither *Z. ramosa* nor *Z. tunicata* results from the recombination of latent factors, or cryptomeres. Had the first appearance of *Z. ramosa* and *Z. tunicata* been the result of the fortuitous combination of factors, or cryptomeres, it would seem to follow that when the new combination was crossed with the parent stock these factors should have been again redistributed and the combination necessary to bring the new characters again into expression should have occurred much less frequently than the observed one in four individuals.

Both of these variations may also be considered either as examples of homœosis (Leavitt, 1909) or as metaphanic variations (Cook, 1915).

The transference of long glumes and the branched conditions from tassel to ear might be looked upon as excellent examples of homœosis. There is, furthermore, a large series of abnormalities that occur in the terminal inflorescence of full-tunicate plants that lend themselves to this interpretation. Spikelets develop into branches, glumes develop into pistils, lodicules develop into glumes, and so on.

On the other hand, it is difficult to think of the nicely graded length of the branches of the tassel of *Z. ramosa* as a translocation of characters. This character is better explained as a metaphanic variation or loss of differentiation, an explanation that would also apply to the branched ear of *Z. ramosa* and the lengthened glumes on the ears of *Z. tunicata*. But here the variation rather overshoots the mark; for, instead of the glumes of the ear being intermediate in length between glumes normal to the ear and the tassel, the glumes of the tunicate ear much exceed normal tassel glumes, and the tassel glumes are themselves much elongated. Neither do the other abnormalities of *Z. tunicata* appear to be typical metaphanic variations, since many of them are repetitions and accentuations rather than intermediate expressions.

There is a sense in which homœotic as well as metaphanic variations may be viewed as a loss or partial loss of differentiation. In typical metaphanic variations the normal specialization of parts is replaced by organs of intermediate form that may be taken to represent an unspecialized ancestral condition. In homœosis instead of the normal specialization certain cells exhibit the characters and potentialities of cells belonging to an entirely different part of the individual. In either instance this loss of specialization or the more or less complete return of cells to an earlier and less specialized condition may be viewed as a reversion.

After concluding that *Z. ramosa* and *Z. tunicata* may be classed as reversions, it is still possible to look upon them as mutations. Both are wide departures from the parent type, and the evidence is that both attained this departure at one step, so far as visible variations are concerned.

With *Z. ramosa* the case is simple. It is a recessive mutation and the dominance of normal maize is complete. The inheritance of *Z. tunicata* is somewhat more complicated. If the interpretation advanced above is correct, and the homozygous form is practically sterile and the common forms heterozygous, *Z. tunicata* would constitute a dominant mutation in which the dominance is imperfect.

The characters separating the two mutations are sharply contrasted. In *Z. ramosa* there is no tendency for the glumes to be elongated more than in normal maize. In tunicate maize the branches of the tassel are no more numerous than in normal maize; and, although the spikelets are much enlarged, the transition from branches to pairs of spikelets is as abrupt as in normal maize. When ears develop on full-tunicate plants, there may be branches near the base of the ear, but these are not at all



homologous to the branches of *Z. ramosa*. The branches in the ear of *Z. tunicata* are proliferated spikelets, while the branches of a *ramosa* ear are, like the branches of the tassel, divisions of the main axis with no evidence of a subtending bract.

#### DESCRIPTION OF THE HYBRID

The cross between *Z. tunicata* and *Z. ramosa* was made at Lanham, Md., in 1914. The female parent was a plant of *Z. ramosa* grown from seed supplied the Department of Agriculture by Dr. Gernert. The male parent was a plant of a tunicate strain developed by Mr. H. J. Sconce. The parent ear was what is here designated as half tunicate.

Nine first-generation plants of this cross were grown at Chula Vista, Cal., in 1915. Of these, four were tunicate and five normal, indicating the heterozygous nature of the half-tunicate parent plant. The tunicate plants were all half tunicate, and no trace of the *ramosa* characters could be seen.

Five self-pollinated first-generation ears were selected for planting in 1916. Three of these ears were tunicate and two normal. The three tunicate ears all showed about the same development of the tunicate character. The seeds were all well covered by the glumes, but the longest glumes did not exceed 30 mm.

The second generation was grown in 1916 at Lanham, Md. Four hundred and eight plants matured, 326 from the three tunicate ears, and 82 from the two nontunicate ears of the first generation.

The progeny of the nontunicate or normal  $F_1$  plants may be dismissed with the statement that the  $F_2$  plants showed segregation into normal and *ramosa* in the ratio of 3 to 1, the numbers being 65 normal and 17 *ramosa*. In none of these plants was there evidence of tunicate characters.

The first impression to be gained from the descendants of the tunicate plants as they came into flower was that there was a completely heterogeneous mixture of the characters of the two parents, together with many new monstrosities. On closer examination it soon became evident that there was one clearly defined group of plants having all the characters of normal maize. It was also possible to distinguish many plants with characteristic *tunicata* or *ramosa* tassels. Among the latter types, however, there were many intermediates, and in addition there was an entirely new type of inflorescence. In this new type the branching habit was developed to a grotesque extreme. As soon as branches formed these again branched. This division continued until the end of the growing season when the tissue was still in an embryonic condition, and nothing resembling floral or foliar organs was formed. The result was a white succulent mass (Pl. 17, 18). This peculiar formation occurred in both lateral and terminal inflorescences, though it was much more common in the former, and in terminal inflorescences it was usually confined to the basal branches.



This abnormality is similar, if not identical, with an abnormality discovered by Blaringhem (1907) in a strain of *Z. tunicata* and termed by him "cauliflower." To judge from Blaringhem's description and plates, the chief differences between his specimens and ours—and these may be only of degree—are that in his examples the disturbance did not extend to the entire inflorescence, and the ultimate ramification terminated in microscopic floral organs, while in ours no sign of floral organs was developed.

Before an examination of the pistillate inflorescences was possible the growing plants were numbered and classified with respect to the character of the tassel. The classification was made on the general appearance of the tassel, and the following classes were recognized: Normal, half tunicate, full tunicate, *ramosa*, and *tunicata-ramosa*, the last class comprising those plants in which both *tunicata* and *ramosa* characters could be recognized, and frequently with more or less tendency to "cauliflower."

There was no occasion for doubt regarding the plants referred to the normal class. The presence of the *tunicata* character was also unmistakable, but the distinction between half and full tunicate was not always easy to make. In the whole field there were three plants recorded as intermediate between half and full tunicate. The plants referred to *ramosa* formed a fairly distinct class, though it was evident that in many of the plants the tassels were more dense with longer glumes and more nearly pendent than was normal to pure *ramosa*, suggesting the presence of *tunicata* characters. There was, thus, some intergradation between the *ramosa* plants and those classed as *tunicata-ramosa*. This uncertainty was dispelled when the ears were later examined, the *ramosa* and *tunicata-ramosa* classes proving to be completely discontinuous.

#### GAMETIC COMPOSITION

The numbers in which the various classes of plants occur are capable of explanation by the assumption of a comparatively simple gametic composition. The terminology here used is to assign a letter to the dominant member of each allelomorph and the same letter, primed, to the recessive member. To those who are accustomed to the presence and absence method of notation it will only be necessary to look upon the primed letters as the absence of the factor, usually designated by a lower-case letter. The custom followed by many workers of assigning the unmodified letter to the factor as it exists in the wild or unmutated form is impracticable in agricultural plants. Since we have not this base line, the unmodified letter is assigned to the dominant member.

On this basis, beginning with the dominant form, full-tunicate plants may be assigned the gametic composition  $TTRR$ ,  $T$  representing the tunicate factor and  $R$  the dominant allelomorph to the *ramosa* factor. *Ramosa*, the other parent, would then be  $T'T'R'R'$ . Ordinary maize with

respect to these characters would be  $T'T'RR$ . Half-tunicate plants, such as the male parent of the hybrid, would be  $TT'RR$ . In a cross between such a plant and *ramosa* the first generation would consist of two kinds of plants,  $TT'RR'$  and  $T'T'RR'$ . Since all contain  $R$ , which is dominant, none would be *ramosa*. One half being heterozygous, for  $T$  would be half-tunicate, the other half would be nontunicate or normal, being homozygous for  $T'$ . Since the tunicate plants of the first generation are heterozygous for both  $T$  and  $R$ , the selfing of such individuals would give all possible combinations (Table I).

TABLE I.—Gametic composition of the hybrid between *Zea ramosa* and *Zea tunicata*

**PARENTS** ..... *Zea tunicata*.

**P<sub>1</sub>**

**Gametes**.....*TR T'R*

*Zea ramosa*.

*T'R'*

**F<sub>1</sub>**

**Zygotes**.....Half tunicate

**Gametes**.....*TR, TR', T'R, T'R'*

**Normal**

*T'R, T'R'*

	<i>TR</i>	<i>TR'</i>	<i>T'R</i>	<i>T'R'</i>
<i>TR</i>	$\frac{TR}{TR}$ Full tunicate	$\frac{TR'}{TR}$ Full tunicate	$\frac{T'R}{TR}$ Half tunicate	$\frac{T'R'}{TR}$ Half tunicate
<i>TR'</i>	$\frac{TR}{TR'}$ Full tunicate	$\frac{TR'}{TR'}$ <i>Tunicata-ramosa</i>	$\frac{T'R}{TR'}$ Half tunicate	$\frac{T'R'}{TR'}$ <i>Tunicata-ramosa</i>
<i>T'R</i>	$\frac{TR}{T'R}$ Half tunicate	$\frac{TR'}{T'R}$ Half tunicate	$\frac{T'R}{T'R}$ Normal	$\frac{T'R'}{T'R}$ Normal
<i>T'R'</i>	$\frac{TR}{T'R'}$ Half tunicate	$\frac{TR'}{T'R'}$ <i>Tunicata-ramosa</i>	$\frac{T'R}{T'R'}$ Normal	$\frac{T'R'}{T'R'}$ <i>Ramosa</i>

	<i>T'R</i>	<i>T'R'</i>
<i>T'R...</i>	$\frac{T'R}{T'R}$ Normal	$\frac{T'R'}{T'R}$ Normal
<i>T'R'..</i>	$\frac{T'R}{T'R'}$ Normal	$\frac{T'R'}{T'R'}$ <i>Ramosa</i>

**F<sub>2</sub>..**

From the above hypothesis and the behavior of the first generation, we should assume that in  $F_2$  all plants homozygous for  $R'$  would be *ramosa*. All plants either heterozygous or homozygous for  $R$  and homozygous for  $T'$  would be normal. Those heterozygous for  $T$  and with at least one  $R$  would be half tunicate. Those homozygous for  $T$  and with at least one  $R$  would be full tunicate. Since the above conditions are not mutually exclusive, there would be combinations calling for the plants to be *ramosa* and at the same time either full or half tunicate.

There were 326 second-generation plants from the *Z. tunicata* first-generation ears. The observed number compared with the number expected in accordance with the gametic composition assumed above are given in Table II.

TABLE II.—Comparison of observed and expected ratios of the different classes of plants

Number expected out of each 16.	Gametic composition.	Characters of plant.	Expected number.	Observed number.
1.....	<i>T'T'RR</i> .....	Normal.....	61. 2	64
2.....	<i>T'T'RR'</i> .....	do.....		
2.....	<i>TT'RR</i> .....	Half tunicate.....		
4.....	<i>TT'RR'</i> .....	do.....	122. 0	121
1.....	<i>TTRR</i> .....	Full tunicate.....		
2.....	<i>TTRR'</i> .....	do.....	61. 2	61
1.....	<i>TTR'R'</i> .....	<i>Tunicata-ramosa</i> .....		
2.....	<i>TT'R'R'</i> .....	do.....	61. 2	64
1.....	<i>T'T'R'R'</i> .....	<i>Ramosa</i> .....		
			20. 4	16
Total 16.....			326. 0	326

When the character of the ear was considered, all the groups, with the exception of half and full tunicate, were perfectly distinct, with no doubtful individuals.

CORRELATIONS BETWEEN TYPE OF PISTILLATE AND STAMINATE INFLORESCENCES

The degree of correlation between the characters of the staminate and pistillate inflorescences may be judged from an examination of Table III.

TABLE III.—Characters of the staminate and pistillate inflorescences of *F*<sub>2</sub> plants of *Zea ramosa* × *Zea tunicata* hybrid

Character of pistillate inflorescence.	Character of staminate inflorescence.						Total.
	Normal.	Half tunicate, glumes 12-24 mm.	Full tunicate, glumes above 25 mm.	<i>Ramosa</i> .	<i>Tunicata-ramosa</i> .	Staminate inflorescences destroyed.	
Normal glumes, 5 mm.....	64	.....	.....	.....	.....	.....	64
Half tunicate, glumes 10 to 44 mm.....	.....	120	3	.....	.....	.....	123
Full tunicate, glumes above 45 mm. or earless.....	.....	4	49	.....	.....	6	59
<i>Ramosa</i> .....	.....	.....	.....	16	.....	.....	16
<i>Tunicata-ramosa</i> .....	.....	.....	.....	37	23	4	64
Total.....	.....	.....	.....	.....	.....	.....	326

NORMAL PLANTS.—It will be seen that among normal plants the correlation is perfect. All plants classed as normal by the tassels proved to have normal ears, and vice versa. The number of normal plants was 64; the expected number 61.2.

HALF-TUNICATE PLANTS.—As previously stated, the distinction between half- and full-tunicate plants is not sharp. If one relies on the gen-

eral appearance, there is seldom any doubt regarding the class to which a plant should be referred; but when the differences are formulated, there is some overlapping. The most obvious tassel characters are the long glumes, the pendent tassel, and the presence of pistillate flowers. Of these, the length of glumes appeared to be the most significant, and was the only one systematically recorded. When the glumes are over 25 mm. long, the tassel in most instances obviously belongs to the full-tunicate class. In classifying the ears, the length of the glumes is also the best character. The dividing line here falls on 45 mm.

There were but three half-tunicate plants in which the length of the staminate glumes exceeded 24 mm. There was, however, no perceptible correlation between the length of glumes in the male and female inflorescences among the half-tunicate plants. One hundred and twenty-three plants were referred to this class. The expected was 122. The nearest approach to an intermediate between half-tunicate and normal plants is shown in Plate 19.

**FULL-TUNICATE PLANTS.**—Fifty-three plants were classed as full tunicate. Of these, 12 produced no ear. Of the remainder, all but 4 had staminate glumes at least 25 mm. long. There is, then, almost a perfect correlation between the type of tassel and the type of the ear; but here again there is no correlation inside the group.

Even the group of plants that were earless did not differ from the plants with ears with respect to the length of the staminate glumes.

There were, in addition, 6 full-tunicate plants with tassels destroyed through accident, making a total of 59 plants in this class. The expected number was 61.2.

**RAMOSA PLANTS.**—Sixteen plants with pure *ramosa* ears all had *ramosa* tassels. The expected number was 20.4.

**TUNICATA-RAMOSA PLANTS.**—There were 60 plants that exhibited both *ramosa* and *tunicata* characters. Of these, 23 exhibited the characters of both parents in the tassel as well as the ear. The remaining 37 plants all had *ramosa* tassels in which no tunicate characters were obvious (Pl. 20, A).

Of the 23 plants which exhibited both *ramosa* and *tunicata* characters in the tassel, 19 had cauliflower ears (Pl. 20, B), 3 showed mixtures of cauliflower and tunicate tendencies, and 1 produced no ear.

In the 37 plants which showed no tunicate character in the tassel, 2 produced cauliflower ears, 11 showed mixtures of cauliflower and tunicate, and 24 were both branched and tunicate without cauliflower (Pl. 21).

In addition to the above, there were 4 plants in which the tassels were accidentally destroyed, making a total of 64 plants in the group. The expected number was 61.2. The nature of the plants combining the characters of both parents is shown in Table IV.

TABLE IV.—Characters of staminate and pistillate inflorescences of the *tunicata-ramosa* group of  $F_2$  plants of *Zea ramosa*  $\times$  *Zea tunicata* hybrid

Character of pistillate inflorescence.	Character of staminate inflorescence.				
	Ra- mosa.	Without cauli- flower.	Partial cauli- flower.	Completely cauli- flower.	Total.
<i>Ramosa</i> .....					
Without cauliflower .....	24				24
Partial cauliflower .....	11		3		14
Completely cauliflower .....	2		19		21
Aborted .....			1		1
Total .....	37		23		60

If the second-generation plants are examined for each of the parental types separately, there is seen to have been a simple 1 to 3 segregation in both instances. One-fourth of the total number of plants are *ramosa* and three-fourths non-*ramosa* (observed, 79 to 247; expected, 81.5 to 244.5). One-fourth are nontunicate and three-fourths tunicate (observed, 80 to 246; expected, 81.5 to 244.5). The distinction between half and full tunicate could not be made when these characters were combined with the *ramosa* character. The various combinations of parental characters, occurring as they do in the normal dihybrid ratios, show that the *tunicata* and *ramosa* characters are not genetically correlated.

In addition to the notes on the inflorescences, the height of each plant was recorded. From these measurements it develops that there were consistent differences in the height of the segregated groups. The averages are given below:

Type of plant.	Height (cm).
Normal .....	221 $\pm$ 2.7
<i>Ramosa</i> .....	195 $\pm$ 2.2
Half tunicate .....	195 $\pm$ 2.4
<i>Tunicata-ramosa</i> .....	191 $\pm$ 2.6
Full tunicate .....	171 $\pm$ 2.7

These differences in height can hardly be explained as differences in vigor due to different degrees of heterozygosity of the characters concerned, since the *ramosa* and half-tunicate groups, which are of the same and an intermediate height, are at once the least and most heterozygous of all the groups.

#### ORIGIN AND SIGNIFICANCE OF CAULIFLOWER INFLORESCENCE

In our experiments the appearance of cauliflower in the inflorescence seems definitely confined to plants in which both the *tunicata* and *ramosa* characters are, as it were, endeavoring to come into expression—that is,



to the plants homozygous for the recessive *ramosa* character and either homozygous or heterozygous for the dominant tunicate character.

The intimate relation between the *ramosa* characters and the cauliflower type of inflorescence may have a bearing on the appearance of cauliflower in Blaringhem's strain of *Z. tunicata*. If the two abnormalities are really of the same nature, the possibility is suggested that in Blaringhem's strain there was a re-occurrence of the *ramosa* mutation. Blaringhem had but few plants of this strain under observation; hence, the absence of pure *ramosa* plants would not be remarkable. In the course of our experiments hundreds of plants of *Z. tunicata* have been examined and their abnormalities studied, but nothing resembling the cauliflower type of inflorescence has been found. In the hybrid under discussion, cauliflower is more definitely associated with the *ramosa* than with the *tunicata* characters. The phenomenon itself appears as an accentuation of the branched habit, and while plants with cauliflower ears occurred without exhibiting *tunicata* characters in the tassel, the *ramosa* characters are in all instances fully expressed.

In making this cross between these two variations from normal maize, each of which may be looked upon as a reversion, the hope was entertained that their combination might bring to light still other ancestral characters and help to give us a slightly more definite conception of the ancestors of maize. In this hope of securing direct evidence we were disappointed. When the two characters are forced to come into expression in the same individual, the result is either a mixture of the two characters or a sterile monstrosity which by no stretch of the imagination can be regarded as an ancestral condition.

From the fact that *Z. tunicata* is a dominant variation Blaringhem (1907) concludes that it is a new or progressive mutation without significance in the study of the ancestry of maize. Since in every particular by which *Z. tunicata* departs from normal maize, it does so by replacing the specialized characters of maize with characters common to practically all other grasses, to place so much emphasis on the dominance of the character seems unreasonable.

The phylogenetic bearing of *Z. ramosa* is less obvious, but even here it seems not unwarranted to consider the variation in the nature of a reversion. The partial incompatibility of the two variations may be explained on the assumption that they represent the recurrence of characters from two widely separated ancestors.

#### SUMMARY

*Z. ramosa* and *Z. tunicata* are looked upon as mutative reversions, the one recessive, the other dominant, as compared with normal maize. The result of crossing these two mutants has been to show that both behave as independent Mendelian units. In the second generation there appears (1) normal maize showing none of the characters of either muta-

tion, (2) the recurrence of both parental types in an apparently pure form, and (3) plants combining the characters of both the mutations. In the last group normal expression is inhibited, and the result is frequently the appearance of a totally different type of inflorescence called "cauliflower," which is sterile, the character being abnormal to the extent that the tissue remains in an embryonic condition, the result being a much-branched, white, succulent mass.

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PLATE 13

*Zea tunicata:*

A.—Plant of full-tunicate type.

B.—Plant of half-tunicate type.

(396)



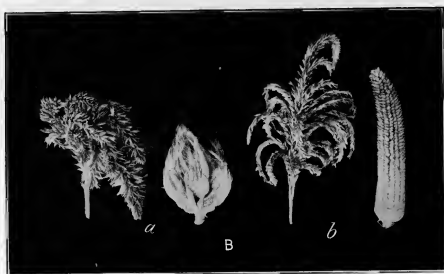
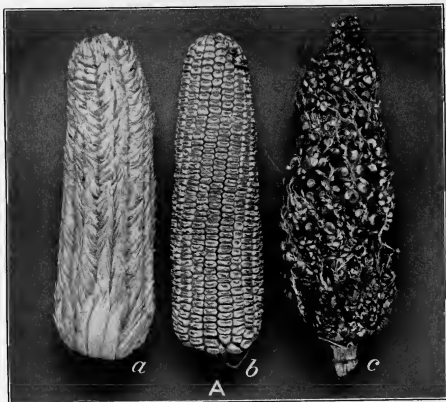




PLATE 14

A.—Pistillate inflorescence of maize. *a*, *Zea tunicata*: Half-tunicate type. *b*, Normal maize. *c*, *Zea ramosa*.

B.—*Zea tunicata*: Terminal and lateral inflorescence. *a*, Full-tunicate type; *b*, half-tunicate type.

PLATE 15

*Zea tunicata:*

Sterile ear of full-tunicate plant.





**PLATE 16**

***Zea ramosa:***

**Staminate and pistillate inflorescence.**



PLATE 17

"Cauliflower" lateral inflorescence borne on F<sub>2</sub> plants of *Zea ramosa* × *Zea tunicata* hybrid.

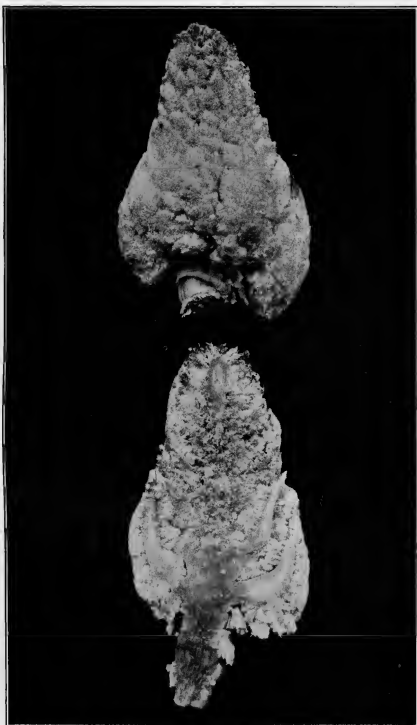




PLATE 18

Terminal inflorescence, cauliflower type of  $F_2$  plant of *Zea ramosa*  $\times$  *Zea tunicata* hybrid.

**PLATE 19**

**Half-tunicate  $F_2$  plant, the nearest approach found to an intermediate between normal and half tunicate.**





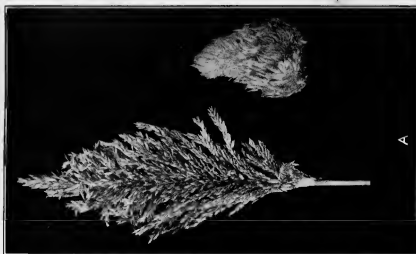
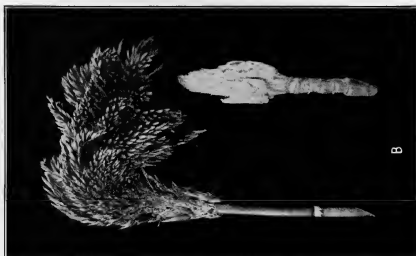


PLATE 20

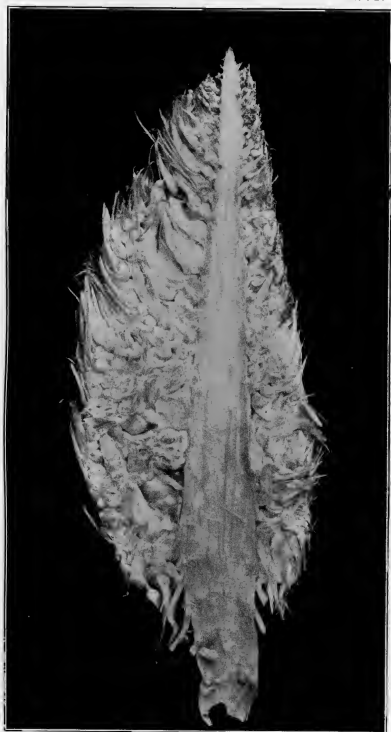
A.—Terminal and lateral inflorescence of  $F_2$  plant, showing the ear both branched and tunicate, and the tassel with only *ramosa* characters.

B.—Terminal and lateral inflorescence of  $F_2$  plant, combining both *ramosa* and *tunicata* characters.

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PLATE 21

Pistillate inflorescence of  $F_2$  plant, showing both the branched and tunicate characters.





# EUPATORIUM AGERATOIDES, THE CAUSE OF TREMBLES<sup>1</sup>

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## INTRODUCTION

In the mountainous sections of North Carolina considerable losses of domestic animals annually occur from a malady known as trembles. This disease is said to be transmissible to man through the ingestion of milk, certain milk products, or flesh of animals affected with trembles, and is known by physicians as milksickness. Since trembles is of so much economic importance to the live-stock interests of the State and since the investigations dealing with its etiology contain such diverse conclusions, a study of this disease was begun during 1916. It is deemed advisable to present at this time the considerable body of data which has accumulated relative to the relationship between *Eupatorium ageratoides* (Pl. 22) and trembles and to reserve for future investigation many important considerations which are as yet unknown or lack conclusive data. Data on certain other phases of the problem have already been secured, but a report of this part of the investigation is reserved for future publication.

## HISTORICAL REVIEW

A very large number of papers dealing with milksickness, or trembles, have appeared in the hundred or more years during which the disease has been known. Most of these articles have been published in the various medical journals, and references to the most important of them are given in several of the more recent investigations (1, 2, 3, 4, 5, 6).<sup>3</sup> These recent papers, furthermore, contain a brief résumé of the important findings of the earlier students of this disease, so that it is necessary for the present purpose to review only the pertinent facts relative to the causes which have been regarded as productive of trembles. In general, these etiological factors may be placed in one of three groups—namely, mineral poisons, poisonous plants, and bacterial parasites.

Arsenic, lead, and cobalt are among the minerals which have been suspected of being the cause of the trouble, but these charges have been disproved.

Among the poisonous plants whose ingestion is regarded as the cause of the trouble are *Rhus toxicodendron*, *Eupatorium ageratoides*, *Lobelia inflata*, and *Bigelovia (Isocoma) heterophylla*. Moseley (4) was of the

<sup>1</sup> Published with the permission of the Director of the North Carolina Agricultural Experiment Station.

<sup>2</sup> Grateful acknowledgment is hereby made of the kindly assistance in this work of our colleagues Mr. Earl Hostetler and Dr. J. I. Handley.

<sup>3</sup> Reference is made by number to "Literature cited," p. 404.

opinion, from experiments in which *E. ageratoides* was fed to various animals, that this weed was the cause of the disorder. His results were not entirely convincing, however, and in criticism of them Crawford (1, p. 15) says:

It can not be said that Moseley has even proved *Eupatorium ageratoides* to be a poisonous plant, much less the cause of "trembles."

When, in the summer of 1906, about 50 head of cattle died of trembles near Minooka, Ill., an investigation of *E. ageratoides* was undertaken by the Office of Poisonous Plant Investigations of the United States Department of Agriculture, since it was the popular belief that this weed was the cause of the trouble. Aqueous extracts were prepared from dried plants and plants preserved in water to which a small amount of chloroform had been added. These extracts were fed or injected subcutaneously into rabbits, cats, and dogs. An aqueous extract from the ash of dried plants was also administered to rabbits. Fifty-eight gm. of fresh plants were, furthermore, fed to a lamb weighing 25 kgm. without the production of symptoms of trembles. In summarizing the results of these experiments, Crawford says (1, p. 19-20):

It certainly can not be said that it has been proved that milksickness is due to any constituent of *Eupatorium ageratoides*. \* \* \*. Again severe epidemics have occurred in winter when the foliage has disappeared, which would tend to exclude the higher, nonevergreen plants as the cause of this disorder. In fact, all the evidence in hand is against the causation of this disease by such plants.

Subsequent publications by Moseley (5, 6) advance the claim that aluminium phosphate found to be present in leaves and stems of *E. ageratoides* is the active toxic principle. Animals fed on this weed or on food in which aluminium phosphate was mixed were found to void aluminium phosphate in the milk and urine. The blood of these animals and certain organs were, moreover, found to contain aluminium phosphate. The stems of rayless goldenrod (*Isocoma heterophylla*) were also found to contain aluminium phosphate; and when this weed, too, was fed to rabbits it produced symptoms similar to those following the feeding of *E. ageratoides* or aluminium phosphate. Since aluminium phosphate is insoluble in water, this accounts, as explained by Moseley, for Crawford's failure to produce poisoning in the experiments in which aqueous extracts of *E. ageratoides* were used. His criticism of the experiment in which 58 gm. of fresh weed were fed to a lamb weighing 25 kgm. is that this quantity would probably not be fatal to a full-grown rabbit.

Experiments conducted by Jordan and Harris (2, 3) in New Mexico and Texas, where this disease is present, but where *E. ageratoides* does not grow, indicate that the disease is of bacterial origin. Trembles developed in rabbits, guinea pigs, dogs, and calves by inoculation with a spore-forming organism which the authors described as *Bacillus lactimorbi*. This organism was present in the milk and butter of cows affected with trembles, in the feces of nonfatal cases in man, in certain parts of the

bodies of affected sheep and horses, and in the soil in regions where milksickness prevails. Taken as a whole, however, the experiments were far from decisive in showing that *B. lactimorbi* is the etiological factor in the production of trembles, or milksickness.

#### METHODS OF EXPERIMENTATION

Since *E. ageratoides*, commonly called "white snakeroot," does not grow in the vicinity of Raleigh, N. C., where the experiments were conducted, it was arranged to secure daily shipments of the green weed from Shooting Creek, N. C., where *E. ageratoides*, locally known as "richweed," grows luxuriantly, especially in shady situations. Since this place is about 300 miles distant from Raleigh, the weed used had been cut about 48 hours prior to its arrival at Raleigh. The weed was fed twice daily to sheep kept singly in small pens in a sheep barn. A maintenance ration of some dry concentrate was given in addition to this green food. The animals used were selected from the experimental flock of grade ewes, all of which were in a healthy condition and, with the exception of those used in the experiments, remained so. The flock number of each individual was retained and is used subsequently in reporting the experiments with the several animals. No case of trembles had ever appeared in this or any other of the Station flocks prior to or during the course of these experiments.

Each animal was weighed when it was placed on the experiment and at time of death. Beginning with experiment 3, in addition to the initial weighings subsequent weighings were made at 3-day intervals until the experiment was concluded or until death resulted. At first the grain and weed were fed separately; but, since the animals either avoided eating any of the weed or ate only sparingly of it, the weed was passed through an ensilage cutter and then mixed with grain before being fed. A daily account was kept of all of the food which was refused by each animal, and these data were employed in approximating the total amount consumed during the course of the experiment. The quantity of *E. ageratoides* eaten by each animal could only be approximated, since the weed and grain refused were mixed and since some loss of weight was due to desiccation. Post-mortem examinations were made of ewes 10, 11, 14, 27, 161, and 169. With the exception of ewe 169, the post-mortem examination showed no evidence that death resulted from any other cause than the feeding of *E. ageratoides*. Post-mortem examinations were not made of the other animals, because the external symptoms were clearly those which characterize trembles.

#### SYMPTOMS OF TREMBLES

Since the possibility exists that trembles in animals may develop from causes other than the feeding of *E. ageratoides* and that the symptoms may differ somewhat from those resulting from the ingestion of this

weed, a brief account of the symptoms observed in the experiments with sheep is pertinent. Considerable individual variation exists in the different animals, both in the period elapsing until the first symptom of trembles is apparent and in the period following until death ensues. Some were sick as early as three days after being placed on the experiment, and no effects were apparent for three weeks in other cases. Sheep usually live three or four days after the disorder is first noticed. Some remain alive, however, for nearly two weeks, and one animal characteristically affected entirely recovered. The feeding of *E. ageratoides* to this animal, however, was discontinued as soon as trembling was noted.

Sheep in the early stages of the disease are sluggish and lie quiet unless urged to rise. They may refuse to eat, or the appetite may be quite normal. There is generally a very considerable decrease in weight, most of which occurs in the last two or three days preceding death. Respirations are accelerated and somewhat labored. A marked stiffness of the legs and ataxia characterizes the movements in walking. If after a day or two the animal is made to stand for a few minutes or is driven a few yards, muscular spasm, especially in the limbs, is evident. The sheep then stands with hind limbs placed well under the body (Pl. 23, A) and all feet spread apart laterally. In this posture the back is bowed, the neck outstretched, and the head lowered. Within a few seconds the quivering spreads over the entire body, increases in intensity, and becomes a violent, involitional tremor (Pl. 23, B). This is accompanied by slight, intermittent, tetanic contractions of the musculature of the limbs. At this stage of trembling ataxia is very pronounced, and the animal is unable to stand (Pl. 24, A). It drops quickly into the normal resting posture (Pl. 24, B), whereupon the trembling immediately ceases. If the sheep is made to rise after it has lain down for a few moments, a second spasm of trembling ensues, with a repetition of the symptoms as described. Trembling may recur repeatedly every time the animal is made to stand. The quiescent period is shortened, however, after each spasm of trembling and may begin as soon as the animal is placed on its feet.

#### RESULTS OF EXPERIMENTS

EXPERIMENT 1.—Three ewes, No. 11, 26, and 10, were used in experiment 1, which was designed to determine whether harmful effects follow the feeding of *E. ageratoides*. This experiment was begun on June 17 and closed on August 2. However, from June 22 to July 6 and from July 18 to July 28 it was impossible to secure the weed. During these periods the animals were grazed on Bermuda grass pasture. Except during the two periods mentioned, a liberal supply of white snakeroot was fed just as it arrived from the point of shipment. In addition, a maintenance ration of grain was fed in a separate trough. Neither the weeds



nor the grain were weighed in this experiment. Initial and final weights of each animal were recorded.

In the period between June 17 and July 16 ewe 11 was fed on *E. ageratoides* and grain for an aggregate of 15 days. A typical case of trembles had developed by July 16, and death occurred two days later. Food was refused during these two days, and there was a decrease in weight from 102 to 77 pounds during the 29 days of intermittent feeding.

Ewe 26 was given a ration of snakeroot and grain for 22 days, between June 17 and August 2. No symptoms of trembles developed during this period. The initial weight of this animal was 91 pounds, and the weight at the time the experiment was discontinued was 74 pounds.

The control ewe, No. 10, was maintained on pasture alone from June 17 to July 28. On July 28 she was put in a pen and was given a ration of *E. ageratoides* and grain until her death, which occurred on August 2. This ewe trembled only slightly, was very weak and emaciated, and lost 20 pounds during the experiment. Although the symptoms were not as marked in this case as in ewe 11, yet all conditions indicated that death was due to trembles.

EXPERIMENT 2.—Three ewes, No. 14, 23, and 26, were employed in experiment 2. This experiment was planned to confirm the results secured in experiment 1. Since the animals used in experiment 1 had refused to eat any considerable quantity of snakeroot when it was fed separately, it was decided to pass the weed through an ensilage cutter and mix it with an equal quantity by weight of grain. One pound of this mixed feed was given each animal twice daily.

On the sixth day after ewe 14 was placed on the experiment she had developed trembles and died on the following day. Her initial weight was 80 pounds, and her weight at death was 73 pounds.

The first symptom of trembles in the case of ewe 23 was noted 19 days after the experiment was begun. A well-defined case of trembles developed in this animal, and she died 6 days after the first symptoms were noticed. Her weight when feeding was begun was 70 pounds, and there was a loss in weight of 8 pounds during the 25 days.

Since ewe 26 had shown no ill effects from the feeding of *E. ageratoides* in experiment 1, she was used in this experiment. It will be recalled that the weed was fed separately and was not ground in the first experiment. Ewe 26 had eaten only sparingly of the weed in this experiment. However, after 16 days' feeding with the mixed ration a very typical case of trembles developed. The feeding of the weed was therefore discontinued and she was put on pasture.

EXPERIMENT 3.—In this experiment ewes 12, 7, 29, 27, and 19 were fed the mixed ration to determine the amount of weed and the length of time required to develop trembles. Table I shows clearly the variation that exists with reference to these two points.



TABLE I.—Results of feeding *Eupatorium ageratoides* to sheep—Experiment 3

Ewe No.	Initial weight.	Experiment begun—	Feeding discontinued—	Days before death occurred.	Weight at death.	Feed consumed.	
						Grain.	Weed.
	<i>Pounds.</i>				<i>Pounds.</i>	<i>Pounds.</i>	<i>Pounds.</i>
12.....	74	Aug. 5	Aug. 18	<sup>a</sup> 13	.....	5.5	11.5
7.....	86	...do....	Sept. 1	27	61	13	9
29.....	81	...do....	Aug. 21	16	55	8	10
27.....	70	...do....	Aug. 10	5	56	2.25	4.5
19.....	113	...do....	Aug. 23	18	89	8	11.25

<sup>a</sup> Feeding discontinued after 13 days.

Ewe 12 was taken off the experiment on August 18, at which time she was affected with trembles. She had lost only 1 pound during these 13 days. It will be noted that the amount of weed required to cause trembles in these five animals varied from 4½ to 11½ pounds and the range in time from 5 to 27 days.

EXPERIMENT 4.—In order to determine whether or not trembles is infectious, ewes 26 and 12 were put in a small Bermuda grass lot on August 18. Two healthy ewes from the flock were put in the same lot and all were fed grain in the same trough. It will be recalled that both ewe 26 and ewe 12 had typical cases of trembles when their feeding in experiments 2 and 3, respectively, was discontinued. Ewe 26 died on August 19 and ewe 12 still trembled a week afterwards. However, she finally recovered fully. Neither of the other two ewes had developed any symptom of trembles when the experiment was discontinued on September 4, and both subsequently remained normal.

EXPERIMENT 5.—This experiment was designed to determine the length of time that *E. ageratoides* must be fed to sheep when, after a certain number of days, the usual grain ration and pasturage are given. Two animals were therefore fed for three days on a mixture of equal parts of ground weed and grain and were then put on pasture. Two others were fed for six days before being placed on pasture and two others for nine days, after which they were put on pasture. Table II contains the essential facts in this experiment.

TABLE II.—Results of feeding *Eupatorium ageratoides* to sheep—Experiment 5

Ewe No.	Initial weight.	Final weight.	Days on experiment.	Feed consumed.	
				Grain.	Weed.
	<i>Pounds.</i>	<i>Pounds.</i>		<i>Pounds.</i>	<i>Pounds.</i>
169.....	83	78	3	1.5	1.5
171.....	85	80	3	1.5	1.5
162.....	96	82	6	5.5	5.5
168.....	89	79	6	4.25	4.25
161.....	102	62	9	6.75	6.75
170.....	105	92	9	5.5	5.5

Ill effects followed only in the cases of ewes 169 and 161, the former dying 8 days and the latter 11 days after being taken off the experiment. Since ewe 169 evidenced no marked symptoms of trembles, a post-mortem examination was made which showed that stomach worms (*Hemonchus contortus*) may have been a contributory cause of her death. Ewe 161, however, developed a typical case of trembles and is the animal represented in the accompanying illustrations (Pl. 23, 24).

EXPERIMENT 6.—Since certain sodium compounds have been suggested as antidotes for trembles, two sheep were given definite quantities of common stock salt and one baking soda along with the mixed ration of *E. ageratoides* and grain. This experiment was conducted between September 18 and October 20. The data on these three animals are presented in Table III.

TABLE III.—Results of feeding *Eupatorium ageratoides*, together with salt or soda, to sheep.—Experiment 6

Ewe No.	Initial weight.	Placed on feed—	Date of death.	Days before death resulted.	Weight at death.	Feed consumed.	
						Grain.	Weed.
	<i>Pounds.</i>				<i>Pounds.</i>	<i>Pounds.</i>	<i>Pounds.</i>
21.....	121	Sept. 18	Sept. 29	11	.....	8	5.25
28.....	96	...do....	Oct. 7	19	73.5	15	10.25
37.....	118	Oct. 2	Oct. 20	18	78.5	10	6

Ewe 21 consumed 8 ounces of salt and ewe 37 ate 12 ounces during the periods of 11 and 18 days, respectively, in which they were on the experiment. Ewe 28 ate with her feed 30 ounces of baking soda.

EXPERIMENT 7.—Since it has been claimed that aluminium phosphate causes a disorder similar to that following the feeding of white snakeroot, two ewes, 166 and 167, were fed aluminium phosphate for a period extending from September 9 to November 17. During this time each ewe was fed 412 gm. of aluminium phosphate ( $\text{AlPO}_4$ ; Baker's, C. P.) mixed with 68.5 pounds of grain, this being supplemented with 138 pounds of alfalfa hay. The daily amounts of aluminium phosphate given were gradually increased from 2 to 16 gm.

At no time during this period of 69 days were these ewes observed to manifest any symptoms of trembles. The initial weight of ewe 166 was 80 pounds, and her weight at the close of the experiment was 91 pounds. The initial and final weights of ewe 167 were 90 and 93 pounds, respectively.

#### SUMMARY

(1) *Eupatorium ageratoides*, commonly known as white snakeroot and locally known in North Carolina as richweed, had previously been claimed by Moseley to cause trembles in animals. This claim has been substantiated by experiments with sheep in which green plants of *E.*

*ageratoides* were fed during the months of June, July, August, September, and October, 1916.

(2) Fifteen cases of trembles in sheep have been developed from feeding *E. ageratoides*. Fourteen of these resulted fatally, and one of them recovered. Death of one of these sheep was probably due in part to an infestation of stomach worms.

(3) Death resulted in from 5 to 27 days following the beginning of feeding of *E. ageratoides*.

(4) Considerable variation existed in the several ewes, also, with reference to the quantity of weed ingested before trembles appeared.

(5) Indirect evidence against the infectious nature of the disease was secured by failure to communicate trembles from sheep characteristically affected to healthy sheep when they were confined and fed together in a small lot.

(6) Salt and soda in the amounts given along with a ration of grain and *E. ageratoides* were without apparent antidotal effect.

(7) No harmful effect followed the feeding for 69 days of aluminium phosphate mixed with grain and supplemented with alfalfa hay.

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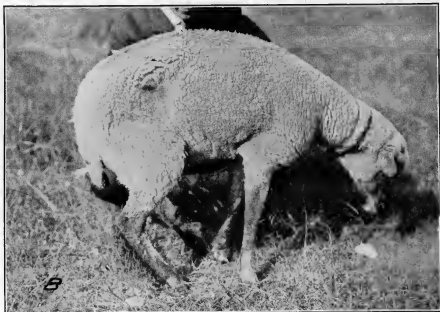
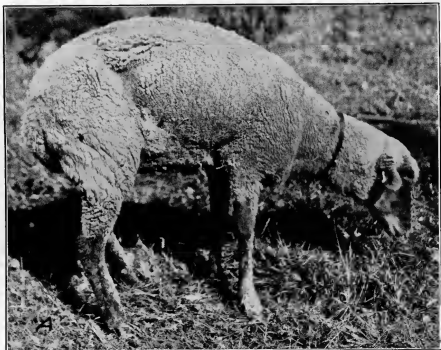
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PLATE 22

*Eupatorium ageratoides*, or white snakeroot.







**PLATE 23**

- A.—Ewe 161 in the characteristic standing posture when trembling is quite violent.
- B.—The same animal with feet spread apart in an effort to stand when the tremors have become more acute.

**PLATE 24**

- A.—At this stage of trembling the animal is unable to stand and is beginning to drop.  
B.—Ewe 161 in the position to which she has dropped after a violent spasm of trembling.



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## A STUDY OF METHODS OF ESTIMATION OF METABOLIC NITROGEN

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### INTRODUCTION

The so-called metabolic nitrogen of the feces is that portion which has an origin other than as an undigested food residue. It consists of residues from the bile and digestive juices, of epithelium and mucus from the digestive tract, and of such products of bacterial activity as have been derived from digested or from digestible nitrogen.

Our reason for wishing to estimate this fraction of the nitrogen of the feces is that it is a factor which must be considered in the determination of the digestibility of protein—a matter of great importance in relation to practical animal and human nutrition.

The plan of this experiment was to feed a basal ration of corn alone to each of five pigs during the first period, and to add to this corn ration in subsequent periods nitrogenous supplements to be used in the comparison of methods. In the selection of these supplements it was our object to choose foods the protein of which would probably be entirely digestible. Those used were milk, blood albumen, and commercial dried egg albumen.

In the comparison of methods of metabolic-nitrogen estimation it was our object to determine which procedure would yield results representing these assumedly entirely digestible protein foods as being entirely digestible—that is, assuming the proteins of milk, for instance, to be entirely digestible, we made an effort to determine which method of estimation of metabolic nitrogen would assign to the protein of milk a digestion coefficient nearest to 100 per cent.

An experimental study involving so much assumption can not yield results of the highest value, but it was our hope that it might assist in the establishment of a useful conventional procedure.



The methods of metabolic-nitrogen estimation compared in this study were the acid-pepsin method, the acid-pepsin and alkaline-pancreatin method, and the alcohol, ether, hot-water, and cold-lime-water method suggested in 1888 by Jordan.<sup>1</sup>

The philosophy of the two methods first mentioned is that by the use of digestive enzymes the nitrogen which has been digested, absorbed, and returned to the feces may be separated from the indigestible nitrogen. In using either of these methods we assume that there is no further digestion, during the course of the estimation, of that part of the food protein which escaped digestion in the alimentary tract of the experimental subject. We have no means of proving the truth of this assumption.

The acid-pepsin method represents stomach digestion alone. The acid-pepsin and alkaline-pancreatin method more nearly follows the physiological process, in that intestinal digestion is also represented. The latter method naturally yields decidedly higher results.

In the Jordan method the treatment with solvents is designed especially for the purpose of washing out bile residues, protein cleavage products and mucin.

The exact procedures followed in the three methods are as follows:

#### ACID-PEPSIN METHOD

Weigh out 5-gm. samples of fresh feces from a weighing bottle; roll up in 9 cm. filter papers, and transfer to 200-c. c. volumetric flasks. Add 100 c. c. of pepsin-hydrochloric-acid solution (made by adding 1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric-acid solution). Shake thoroughly and put into an air bath maintained at 38° to 40° C. Allow the digestion to continue for 24 hours. During the first 6 hours agitate by rotation once each hour; agitate again 1 hour before final removal from the air bath. Arrange funnels with 12.5 cm. fluted quantitative papers, and dry 100-c. c. volumetric flasks. Promptly at 24 hours from the time of starting the digestion remove the 200-c. c. flasks from the oven, cool, fill to the mark with cold distilled water, mix thoroughly, and filter. Determine the nitrogen in 100 c. c. of the filtrate. The result represents metabolic nitrogen.

#### ACID-PEPSIN AND ALKALINE-PANCREATIN METHOD

Weigh 1.5 to 2.5 gm. samples of fresh feces into 150 c. c. Jena beakers. Add 100 c. c. of acid-pepsin solution (1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric acid). Stir thoroughly with a glass rod and place in an air bath maintained at 38° to 40° C. Stir thoroughly once each hour for the first 8 hours. Allow the digestion to continue for exactly 24 hours. Filter immediately through 12.5 cm. fluted quanti-

<sup>1</sup>[Jordan, W. H.] Analytical and experimental methods. Protein digestion. *In* Maine Agr. Exp. Sta. Ann. Rpt. 1888, p. 197. 1889.

tative filters. Wash beakers, filters, and contents until free from acid, with water at a temperature of 40° C.

Return filters and contents to the proper beakers and treat with 100 c. c. of alkaline-pancreatin solution (1.5 gm. pancreatin in somewhat less than 1 liter of water; add 3 gm. of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ); dilute to exactly 1 liter, and mix thoroughly). Return the beakers to the bath and stir thoroughly. Allow to digest for exactly 12 hours. Filter immediately through fluted papers. Wash beakers, filters, and contents thoroughly and repeatedly with hot water, and allow to dry. Transfer the filters and contents to Kjeldahl flasks and determine the nitrogen in the usual manner. Subtract the result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

#### JORDAN'S METHOD<sup>1</sup>

Weigh 2 to 3 gm. portions of fresh feces, and dry at 100° to 105° C.; transfer to extraction capsules and extract with ether for 16 hours. Transfer to 150-c. c. beakers and treat with 50 c. c. of boiling 95 per cent alcohol. Keep at boiling temperature for 10 minutes; decant the alcoholic extract through qualitative filters; wash several times with hot alcohol and once or twice with ether, by decantation. With a camel's-hair brush transfer the residue from the filter papers to the original beakers; add 50 c. c. of hot water and boil for 10 minutes; filter through the same papers used for the last filtration, washing with hot water, by decantation. Wash the residues from the filter papers back into the beakers with 50 c. c. of a saturated solution of calcium hydrate, and let stand for 6 hours; filter through the same filters last used; transfer all the material from the beakers to the filter papers; wash with lime water, and allow to drain. Transfer filter papers and contents to Kjeldahl flasks, and determine the nitrogen. Subtract result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

#### EXPERIMENTAL PROCEDURE

The subjects of this experiment were five Yorkshire barrows of nearly uniform age and weight. The average weight at the end of the first period was 53.85 kgm., and at the end of the fourth, 59 days later, 84.42 kgm., the average daily gain in weight being 518 gm., or 1.14 pounds. They were confined in the metabolism crates illustrated in our previous publications.<sup>2</sup>

<sup>1</sup> Jordan, W. H., *Op. cit.* (Detailed specifications were not submitted in the original publication; the particulars as here stated were arbitrarily assumed.)

<sup>2</sup> Forbes, E. B., Beegle, F. M., and others. A chemical study of the nutrition of swine. *Ohio Agr. Exp. Sta. Bul.* 271, p. 224-261, 3 pl. 1914.

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The experimental periods were of 10 days' duration, separated by 7-day intervals during which were fed the rations of the periods to follow. The feces were marked with carmine.

Table I records the total amounts and nitrogen content of the foods consumed and feces produced.

Table II records the percentages of total nitrogen in the feces and of metabolic nitrogen, as estimated by the three different methods.

Table III records the coefficients of digestibility of the nitrogen of the foods.

TABLE I.—Foods consumed and total nitrogen in foods and feces

Period No. (10 days).	Pig No.	Foods consumed.		Nitrogen in foods.		Weight of feces.	Total nitrogen of feces.	
		Corn.	Supplements.	Corn.	Supplements.			
		Gm.	Gm.	Gm.	Gm.	Gm.	Per cent.	Gm.
I.....	1	18,200	.....	245.700	.....	6,357	0.995	63.252
	2	19,731	.....	266.373	.....	7,553	.889	67.146
	3	20,000	.....	270.000	.....	7,954	.989	78.665
	4	18,000	.....	243.000	.....	5,866	1.005	58.953
	5	16,800	.....	226.800	.....	5,645	1.000	56.450
II.....			Blood albumen.		Blood albumen.			
	1	16,200	730	215.298	83.987	5,737	.888	50.945
	2	18,000	810	239.220	93.191	6,144	.785	48.230
	3	19,000	855	252.510	98.368	6,710	1.006	67.503
	4	17,100	770	227.259	88.589	5,428	.892	48.418
	5	17,100	770	227.259	88.589	5,226	.943	49.281
III.....			Skim milk.		Skim milk.			
	1	17,100	21,400	230.679	111.708	5,857	1.082	63.373
	2	19,000	23,800	256.310	124.236	6,566	.900	59.094
	3	19,000	23,800	256.310	124.236	6,936	1.059	73.452
	4	17,100	21,400	230.679	111.708	5,672	1.066	60.464
	5	17,100	21,400	230.679	111.708	5,529	1.060	58.607
IV.....			Egg albumen.		Egg albumen.			
	1	17,100	770	234.441	87.226	5,129	1.226	62.882
	2	19,000	855	260.490	96.854	5,847	.989	57.827
	3	19,000	855	260.490	96.854	6,263	1.346	84.300
	4	17,100	770	234.441	87.226	5,410	1.177	63.676
	5	17,100	770	234.441	87.226	5,371	1.070	57.470

TABLE II.—Total and metabolic nitrogen of feces (per cent)

Periods (10 days).	Pig No.	Total nitrogen.	Metabolic nitrogen.		
			Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I.....	I	0.995	0.706	0.836	0.473
	2	.889	.619	.760	.418
	3	.989	.701	.834	.468
	4	1.005	.656	.816	.447
	5	1.000	.741	.858	.445
II.....	I	.888	.585	.749	.422
	2	.785	.581	.677	.404
	3	1.006	.773	.857	.446
	4	.892	.641	.738	.368
	5	.943	.712	.820	.380
III.....	I	1.082	.714	.831	.426
	2	.900	.590	.693	.347
	3	1.059	.739	.857	.392
	4	1.066	.704	.814	.416
	5	1.060	.691	.859	.420
IV.....	I	1.226	.802	.965	.525
	2	.989	.670	.761	.451
	3	1.346	.935	1.123	.599
	4	1.177	.729	.949	.505
	5	1.070	.742	.840	.433

TABLE III.—Coefficients of digestibility of nitrogen

Period No. (10 days).	Pig No.	Apparent digestibility. <sup>a</sup>	Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I (corn).....	I	74.26	92.52	95.89	86.49
	2	74.79	92.34	96.34	86.64
	3	70.86	91.52	95.43	84.65
	4	75.74	91.58	95.44	86.53
	5	75.11	93.55	96.47	86.19
II (blood albumen).....	I	105.33	98.48	101.04	102.80
	2	112.96	106.22	102.27	109.18
	3	106.18	105.87	101.57	101.20
	4	107.58	106.22	102.26	102.45
	5	108.22	102.92	101.80	102.21
III (skim milk).....	I	96.42	96.15	95.33	93.50
	2	104.44	99.42	96.61	98.34
	3	100.00	99.63	98.15	94.43
	4	95.97	99.01	96.62	94.81
	5	98.93	95.06	97.34	96.84
IV (egg albumen).....	I	97.09	95.17	95.70	95.09
	2	108.10	101.34	96.08	103.45
	3	91.33	96.23	97.87	92.98
	4	92.20	94.84	98.12	98.25
	5	101.01	97.14	95.33	97.89

<sup>a</sup> On basis of total nitrogen of the feces.

## CONCLUSIONS

The apparent digestibility of the protein of corn, based on the total nitrogen of the feces is about 75 per cent. On account of the existence in the feces of nitrogen of metabolic origin we know that the real digestibility is higher. The acid-pepsin method makes it appear that the real digestibility of the protein of corn is about 92 per cent, and the pepsin-pancreatin method about 96 per cent. Jordan's method gives appreciably lower figures, averaging 86 per cent.

The acid-pepsin method indicates that 70 per cent, the pepsin-pancreatin method 84 per cent, and the Jordan method 46 per cent of the nitrogen of the feces from corn is of metabolic origin.

All of the methods make the nitrogen of blood albumen appear more than completely digestible, even the apparent digestibility being over 100 per cent; thus, the feeding of blood albumen with corn seems to increase the digestibility of the corn protein to an extent more than sufficient to offset the incompleteness of digestibility of the protein of this supplement.

With skim milk the apparent digestibility varies from 95.97 to 104.44 per cent, the average being 99.15. With the acid-pepsin method three out of the five figures average 99.35. In previous work <sup>1</sup> five estimations by this method averaged 99.12. With the pepsin-pancreatin method the results were lower than with the acid-pepsin method. These low results on the supplementary food are reciprocals of the high results on the basal ration of corn.

The proteins of skim milk are made to appear more nearly completely digestible by the acid pepsin method than by the pepsin-pancreatin method or by the Jordan method.

With egg albumen the results varied considerably, but all were high. It would appear that raw, commercial, dried egg albumen is almost perfectly digested by swine.

Important inaccuracy seems to be inevitable in any determination of digestibility of supplementary foods in the usual way, by difference; and no other method seems more satisfactory. This applies equally to computations of real digestibility, and of apparent digestibility (based on total nitrogen of the feces).

The digestion coefficients for protein involved in the feeding standards of our reference works on animal production assume that the nitrogen of the feces is entirely an indigestible food residue. The rough measures afforded by the results of this study indicate that, as applying to the digestive capacities of swine, this assumption underestimates the digestibility of protein by about 20 per cent.

By way of interpretation of the individual variations in the digestion coefficients we would record the fact that pig 1, in Period II, manifested

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<sup>1</sup>Forbes, E. B., Beagle, F. M., and others. *Op. cit.*



a pronounced dislike for the blood albumen. The acid-pepsin method indicates that this pig was less able than others to digest this foodstuff. Also, we would observe that, in response to most insistent demands for food, we gave to pig 5 in Period II a larger allowance of food per unit of body weight than was given to the other individuals. The digestion coefficient for blood albumen, as determined by the acid-pepsin method, with this pig also is low. Further, these two pigs, No. 1 and 5, had the lowest digestion coefficients, as determined by the acid-pepsin method, in the following period, No. III, where skim milk was fed.

In a study of the effects on metabolic nitrogen of storage of the feces in a frozen condition for 20 days, with and without the addition of thymol, compared with air-drying the fresh material, with and without thymol, no significant differences were observed which could be related to these methods of preservation.

In attempting to choose between these methods it seems to us that the acid-pepsin and the pepsin-pancreatin methods give results which are more nearly true than does Jordan's method, since the latter does not digest the bacteria, which may contain large proportions of the nitrogen of the feces and which presumably are more largely the product of digestible than of indigestible protein; but it is idle to attempt close comparisons of such conventional and inaccurate procedures. We have no accurate scientific basis for the determination of the digestibility of protein.





# A NEW STRAIN OF RHIZOCTONIA SOLANI ON THE POTATO

By J. ROSENBAUM, *Mycologist*, and M. SHAPOVALOV, *Agent, Cotton, Truck, and Forage-Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*.<sup>1</sup>

## INTRODUCTION

Investigators heretofore have spoken of strains of *Rhizoctonia solani* Kühn when referring to cultures isolated from different hosts as well as to different isolations from the same host. Thus, Duggar<sup>2</sup> states (p. 423):

The potato is the most interesting of the host plants with respect to the parasitism of *Rhizoctonia* by reason of the many types of disease induced under diverse conditions. The conditions may be in part climatic and, in part perhaps, dependent upon the pathogenicity of the particular strain of the fungus . . .

He further states (p. 442):

Strains do occur, however, evidence of which may persist for some time in the general appearance of the cultures.

Edson<sup>3</sup> says—

Much confusion exists regarding the identity of the various forms, and there is likewise great diversity of opinion as to the pathogenic properties of the members of the group.

Thus, while it is recognized that differences do exist, it has not been shown that it is possible to distinguish the different strains, especially from cultures obtained from the same host, either from their morphology or their growth on various media. Thus, Peltier<sup>4</sup> makes the following statement:

. . . hence, on the measurement of the mycelial cells of *Rhizoctonia Solani*, as on the study of the growth on media, no conclusions can be based in regard to the distinguishing strains of this difficult species.

The purpose of this paper is to present evidence that two strains of *R. solani* are found on the potato (*Solanum tuberosum*), and, further, that it is possible to distinguish these with accuracy from the macroscopic growth on various media, as well as by the more accurate morphological comparisons.

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<sup>1</sup> The writers are indebted to Dr. H. A. Edson, of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, for advice and suggestions during the progress of this work.

<sup>2</sup> Duggar, B. M. *Rhizoctonia crocorum* (Pers.) DC. and *R. solani* Kühn (*Corticium vagum* B. & C.), with notes on other species. *In* Ann. Mo. Bot. Gard., v. 2, no. 3, p. 493-458, 9 fig. 1915. Bibliography, p. 452-458.

<sup>3</sup> Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour. Agr. Research, v. 4, no. 2, p. 151. 1915.

<sup>4</sup> Peltier, G. L. Parasitic *Rhizoctonias* in America. Ill. Agr. Exp. Sta. Bul. 189, p. 372. 1916.

## SOURCE OF MATERIAL

The cultures of *R. solani* on which the following studies are based were obtained from stems and tubers of potatoes grown in Florida and northern Maine. The isolations were made during the summers of 1915 and 1916, so that the comparisons are made with cultures of comparatively the same age. For the sake of convenience they are here designated "R<sub>1</sub>," "R<sub>2</sub>," "R<sub>3</sub>," etc. R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> were isolated in Maine during the summer of 1916 from the base of affected plants. R<sub>4</sub>, R<sub>5</sub>, and R<sub>6</sub> were obtained from the same locality from the inside of potato stems. R<sub>7</sub> was isolated from tubers obtained at Hastings, Fla., in 1915, and R<sub>8</sub> from tubers in Maine during the summer of 1915. Throughout these studies all the strains, from R<sub>1</sub> to R<sub>8</sub>, could not be distinguished, with the single exception of R<sub>5</sub>. In presenting the results, therefore, R<sub>5</sub> will be compared with a representative of one of the other cultures. The cultures from the stems were obtained from plants showing a girdling and hollowing at or near the surface of the ground (Pl. 25, A). This condition appeared to be a secondary stage in a malnutrition trouble briefly described by Edson and Schreiner.<sup>1</sup>

## DISTINGUISHING CHARACTERS

The points of difference between R<sub>5</sub> and the other strains, as here presented, can be grouped as pathological, as shown by inoculation experiments; physiological, as shown by the reactions on different media; and morphological, as shown by the measurements of mycelium, of surface sclerotial cells, and of diameters of germ tubes produced by germinating sclerotial cells.

## PATHOLOGICAL CHARACTERS

Inoculations were made in the field on healthy growing plants. The method of procedure was to wash any dirt from the stems, make a slight incision with a flamed scalpel, and insert a bit of a young growing culture into the wound. The control plants were likewise injured. A number of such inoculations with R<sub>5</sub>, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, etc., and a number of undetermined fungi also isolated from diseased stems, resulted in the production of very pronounced lesions with R<sub>5</sub>. The lesions produced by R<sub>1</sub>, R<sub>2</sub>, etc., were indefinite, as was also the case with all the undetermined fungi. The injured control plants remained healthy. Plate 25, B, illustrates the results of one of the series of inoculations. The three stems to the left were inoculated with R<sub>5</sub>, the next two with R<sub>1</sub> and R<sub>2</sub>, and the last shows the condition of the control.

Inoculations were also repeated in the greenhouse, with results practically similar to those described above.

<sup>1</sup> Edson, H. A., and Schreiner, Oswald. A malnutrition disease of the Irish potato and its control, *In* *Phytopathology*, v. 7, no. 1, p. 70-71. 1917.

Inoculations on the tubers were also made. The method followed was to select clean and healthy tubers, immerse these for 10 minutes in a 1 to 1,000 mercuric-chlorid solution, wash with sterilized water, make a slight incision with a sterile scalpel, and insert a bit of a pure culture in the wound. Control tubers were treated in a similar manner, the agar medium without the fungus being inserted. The lesions produced with any of the strains were never very large, but a very distinct lesion was produced with R5 as compared with the other strain. Plate 26, B, illustrates a tuber inoculated with R5. For comparison a control tuber is shown in Plate 26, C. In several instances the lesion as a result of the inoculation extended to  $\frac{1}{4}$  inch in diameter.

The strain R5, therefore, is not only more pathogenic on the stems than the remaining strains isolated from the potato, but is in fact able to produce a distinct necrosis of the tissues of the potato tuber.

#### PHYSIOLOGICAL CHARACTERS

The various strains were grown on a variety of media. Only those on which R5 has shown any marked distinguishing characteristics will be pointed out here.

**POTATO AGAR.**—On this medium, when grown in test tubes, R5 at the end of a week or 10 days produces a very marked discoloration of the medium. This coloration is dark brown, approaching black. The discoloration, if produced at all by the other strains on this medium, is very much less pronounced and never approaches the intensity of color produced by R5.

**CORN-MEAL AGAR.**—On this medium, when grown in test tubes, R5 produces light-gray, loosely formed sclerotia as compared with the darker, brownish, and more compact sclerotia formed by the other strains (Pl. 26, A). The character is very striking and can be relied upon.

**USCHINSKY'S SOLUTION.**—One hundred cubic centimeters of this solution was poured into 200-c. c. Erlenmeyer flasks and inoculated with small bits of pure cultures of the various strains grown on potato agar. The rate of growth of R5 is far in excess of the remaining strains. At the end of 10 days R5 entirely covered the surface and was growing on the side of the flask, while the growth of the remaining cultures were still below the surface of the liquid.

#### MORPHOLOGICAL CHARACTERS

In holding up against the light some petri-dish cultures on string-bean agar of the various strains, the writers were struck with an apparent difference in the fineness of the mycelial strands of R5 as compared with the remaining strains. A mount made and examined under the microscope revealed a distinct difference in the fineness of the mycelium. This difference in the diameter of R5 and the remaining strains was very evident

microscopically without definite measurements. Subsequent examinations made with the microscope, from cultures of various ages, as well as from a variety of different media, showed this difference constant, the diameter of the mycelium of R5 being in every case smaller than that of the remaining strains. Measurements of R5 and the remaining strains were made from cultures of the same age grown on the same media. The procedure followed was to grow the cultures in petri dishes, and mounts on slides were made a definite distance from the original planting. Fifty measurements were made at random in each case. The results of these measurements show that the diameter of R5 varies from 4.7 to 8.8  $\mu$ , with 7.8  $\mu$  as the average measurement, while the measurements of the remaining strains vary from 10 to 14.0  $\mu$ , with 10.1  $\mu$  as the average. Figure 1 illustrates this difference comparatively, both sections being drawn with the aid of the camera lucida, with the same magnification and from cultures of the same age. That the thickness

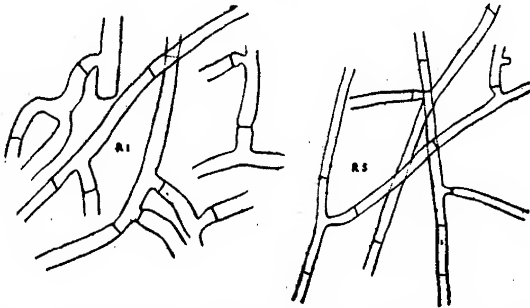


FIG. 1.—Camera-lucida drawings of mycelium of the R1 and R5 strains from the same medium and cultures of the same age.

of the mycelium is a good distinguishing character was confirmed as follows: 19 mounts were made from cultures grown on various media and of varying ages. The R5 was marked at the time of mounting, but in such a way that the person to whom they might be submitted was unaware of any distinguishing marks.

These were submitted to two pathologists of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, with a request that they divide the mounts into two lots, based on the diameter of the mycelium. In every case the mounts of R5 were separated from the remaining strains.

Not only is the diameter of the mycelium of R5 smaller, but likewise the short sclerotial cells enveloping the sclerotia are smaller, as shown in figure 2. One hundred measurements show that those of R5 vary in length from 13.6 to 30.6  $\mu$ , with an average of 21.6  $\mu$ , while the others measure 17 to 61.2  $\mu$ , with an average of 37.5  $\mu$ . In width those of R5 measure from 8.3 to 20.4  $\mu$ , with an average of 12.3  $\mu$ . The other strains measure from 11.9 to 23.3  $\mu$ , with an average of 16.7  $\mu$ . Generally they are also much more regular than those found in the remaining strains, so much so, in fact, that they can be described as "monilia-like." In the case of the others, R1, R2, etc., while occasionally one sees a chain of regular sclerotial cells, this is the exception rather than the rule. It may perhaps be argued that R5 was contaminated with another sterile fungus the mycelium of which resembles that of a species of *Rhizocotonia*, but is of

a smaller diameter. It was not possible to obtain a culture from a single basidiospore, but dilution plates were made and a culture obtained from the germination of a single short typical sclerotial cell. This culture

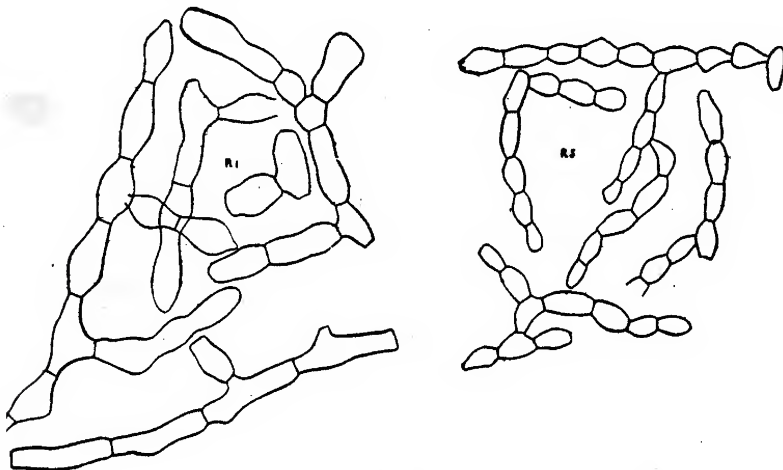


FIG. 2.—Camera-lucida drawings of short sclerotial cells of the R1 and R5 strains from cultures of the same age grown on the same medium.

agreed in all essential characters previously enumerated with the other cultures of R5.

While the mycelium measurements of R5 and all the remaining strains with which the writers worked are distinct, they do show variation, as indicated by the measurements given above. There may also be a question as to the accuracy of random measuring, even from mounts made from the same medium of the same age. A more accurate manner of identification of R5 from the remaining strains consists in the germination of the short sclerotial cells in water and the measurement of the diameter of the germ tubes produced. The mode of germination of the short sclerotial cells of R5 agreed in all essential characters with that of the remaining strains and has been described by Duggar<sup>1</sup> and others. Measurements made of the diameter of the germ tubes produced by R5 and the remaining strains

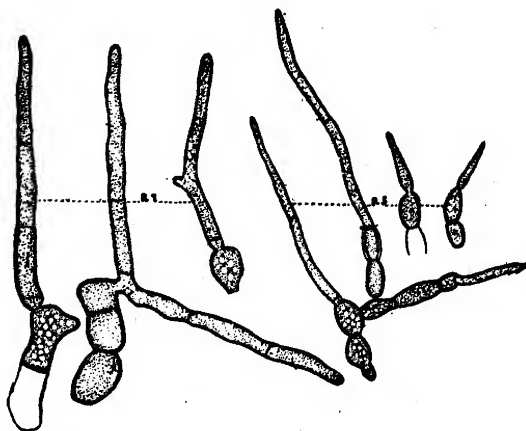


FIG. 3.—Camera-lucida drawings of germinating sclerotial cells of the R7 and R5 strains. The drawing was made at the end of 17 hours, after the cells were placed to germinate in drops of water.

<sup>1</sup> Duggar, B. M. Op. cit.



showed that the germ tubes produced by R5 are constantly smaller than those of the remaining strains. Figure 3 illustrates this relation. The writers consider this character the most reliable in the identification of these strains. In making the measurements of the germ tubes the question arose whether different length germ tubes produce tubes of varying width and also whether the size of the sclerotial cell is correlated with the size of the germ tube. In order to determine this point, 100 measurements were made of the short sclerotial cells, the length of the germ tube, together with the width of same. The results may be summarized as follows: The size of the cell has no apparent effect on the length or width of the germ tube; nor has the length of the germ tube any appreciable effect on the diameter of the germ tube. The width of the germ tubes produced by the germinating enveloping sclerotial cells of R5 vary from 3.4 to 6.8  $\mu$ , with an average of 4.3  $\mu$ , while those of R7 and R4, taken as representatives of the remaining strains, vary from 6.8 to 11.9  $\mu$ , with an average of 8.5  $\mu$ .

Table I summarizes the measurements made in the above comparative studies.

TABLE I.—Comparative measurements of strains R5 and R7 of *Rhizoctonia solani*

	Diameter of mycelium.		Sclerotial cells.				Diameter of germ tubes.	
			Length.		Width.			
	R <sub>5</sub>	R <sub>7</sub>	R <sub>5</sub>	R <sub>7</sub>	R <sub>5</sub>	R <sub>7</sub>	R <sub>5</sub>	R <sub>7</sub>
Minimum.....	$\mu$ 4.7	$\mu$ 10.0	$\mu$ 13.6	$\mu$ 17.0	$\mu$ 8.3	$\mu$ 11.9	$\mu$ 3.4	$\mu$ 6.8
Maximum.....	8.8	14.0	30.6	61.2	20.4	23.3	6.8	11.9
Average.....	7.8	10.1	21.6	37.5	12.3	16.7	4.3	8.5

COMPARISON WITH RHIZOCTONIA CROCORUM (Pers.) DC.—Duggar gives the mycelial measurements of *R. crocorum* (Pers.) DC. as 4 to 8  $\mu$ ; of *R. solani* Kühn, 8 to 12  $\mu$ . According to the measurements of the writers, R5 varies from 4.7 to 8.8  $\mu$  and the common strain of *R. solani* from 10 to 14  $\mu$ . These measurements agree so closely with those of *R. crocorum* and *R. solani* as given by Duggar that there appeared to be need for a closer comparison with *R. crocorum*. Such a comparison was made with exsiccatae material, with material of *R. crocorum* on asparagus collected in Germany by Dr. H. A. Edson, of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, and kindly furnished by him to the writers, and lastly with the descriptions and distinguishing characters as outlined by Duggar.<sup>1</sup> Unfortunately, the original specimens from which the isolations were made were not saved, as at that time it was not known that the strain of *Rhizoctonia* isolated was essentially different

<sup>1</sup> Duggar, B. M. Op. cit.

from the common strain of *R. solani*. It is therefore impossible to state with any degree of accuracy the color of the mycelium and sclerotia on the host, the presence or absence of infection cushions, etc. The writers, however, are reasonably sure that these characters were not those belonging to *R. crocorum*. Aside from the above consideration, its similarity to *R. crocorum* is shown by (1) the close agreement of mycelial measurements, (2) the sclerotia in culture approaching the plectenchymatic type. The differences are shown by (1) the myceliums lacking the typical violet or violet brown pigment in cultures of considerable age on a variety of media, (2) the branchings not being at right angles, though occasionally so, and (3) most important perhaps, the ease with which it is grown in culture on a variety of media contrary to the experience of all former investigators.

A consideration of the above facts lead the writers to believe that R<sub>5</sub> is to be regarded as a distinct strain of the common *R. solani* Kühn as occurring on the potato rather than one of *R. crocorum* (Pers.) DC.

#### SUMMARY

A strain of *R. solani* Kühn, for the sake of convenience temporarily designated as "R<sub>5</sub>," was isolated from potato stems in Maine during the summer of 1916. This strain can be distinguished from the more common *R. solani* by (1) the more pronounced lesions produced when inoculated on injured stems or tubers; (2) the reaction, growth, and character of sclerotia on definite media; and (3) morphologically, by measurements of the mycelium, of the short sclerotial cells, and, lastly, by the measurement of the diameter of germ tubes when the short, or "barrel-shaped," cells enveloping the sclerotia are placed in drops of water to germinate.

PLATE 25

A.—Potato stems, showing the nature of the lesions from which isolations of *Rhizoctonia solani* were made.

B.—Potato stems, showing the results of one series of inoculations. Three stems to the left were inoculated with strain R5, the next two inoculated with R1 and R2, and the stem to the extreme right an injured control. The inoculations were made on August 14, 1916, and the photograph was taken on September 4, 1916.

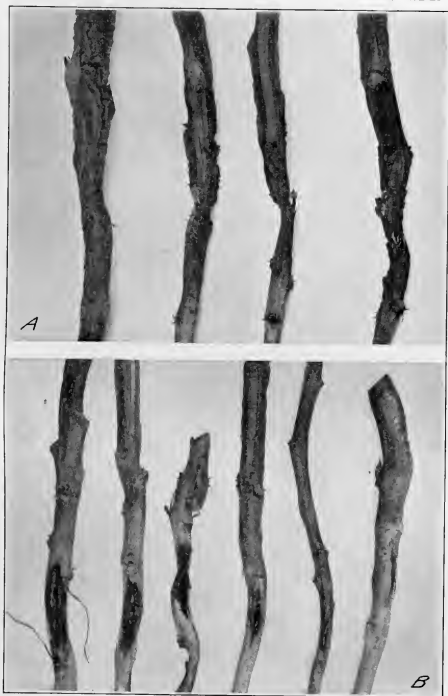




PLATE 26

A.—Reaction on corn-meal agar of R<sub>5</sub>, as compared with the other strains. Cultures were made on November 8, 1916, and were photographed on November 27, 1916. The two test tubes to the left show the character of the growth of strain R<sub>1</sub>, the next two those of R<sub>5</sub>, and the two test tubes to the right those of R<sub>7</sub>.

B.—A potato tuber, illustrating the results of inoculation with strain R<sub>5</sub>. The inoculation was made on November 5, 1916, and the photograph taken on November 15, 1916.

C.—An injured potato tuber (control). Photographed on November 15, 1916.



# A FORM OF POTATO DISEASE PRODUCED BY RHIZOCTONIA

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What appeared to be an undescribed type of potato-tuber disease was observed a few years ago in southern Maine for the first time. Material from this region has been available for study during the present winter (1917). While there is no reason to think that it does not occur in other parts of the State, the writer has not seen any noticeable cases of this trouble in northern Maine, although all of the summer of 1916 was spent in the study of potato (*Solanum tuberosum*) diseases in Aroostook County.

This type of trouble was first recorded in Maine by Morse and Shapovalov.<sup>2</sup> Moreover, after examining a considerable number of potatoes received from various parts of the country as illustrating different types of potato-scab, they were led to make the following statement relative to this form of potato-tuber injury mentioned above:

A critical examination of potatoes from a large number of sources, including those from other States, has convinced the writers that it is fairly common.

While several authors have mentioned scabbing, pitting, cracking, and ulcer formation more or less in association with Rhizoctonia, so far as the writer has been able to ascertain, with the exception of the publication mentioned above, no other references have been made in the literature to this type of scabbing or pitting, or, as the writer has chosen to call it, "dry core" of the potato tuber. Probably one of the nearest references to this type of trouble was made by Rolfs<sup>3</sup> in his description of an experiment with Rhizoctonia. He placed small amounts of hyphæ from pure cultures of the fungus on the surface of small growing potatoes and covered them with sterilized grafting wax. Fourteen days afterward two of these potatoes were examined, and a number of brown spots were found on the inoculated surfaces. Microscopic examination showed that the hyphæ had entered the lenticels and produced small ruptures in the skin. Further on he states that all of the inoculated tubers developed rough surfaces and cracks, but nothing was mentioned that would lead

<sup>1</sup> Thanks are due to Dr. W. J. Morse for materials and for valuable suggestions in formulating this paper.

<sup>2</sup> Morse, W. J., and Shapovalov, Michael. The Rhizoctonia disease of potatoes. Maine Agr. Exp. Sta. Bul. 230, p. 206. 1914.

<sup>3</sup> Rolfs, F. M. Potato failures. A preliminary report. Colo. Agr. Exp. Sta. Bul. 70, p. 11. 1902.

one to believe that anything similar to our type of injury was developed. In a later publication<sup>1</sup> he makes this statement:

European investigation long ago attributed the pitting or scabbing of tubers to the attacks of *Rhizoctonia*. Our experiments and observations also show that its attacks on growing tubers frequently produce deep ulcers. Most of our scab is due to the attacks of this fungus.

Other authors at about this same time observed *Rhizoctonia* hyphæ associated with the so-called scab ulcers, but were of the opinion that this fungus had nothing to do with their formation. However, in no case has the writer been able to find either photographs, drawings, or descriptions that give any adequate idea as to just what is meant by a scab ulcer.

Without a doubt this form of trouble has escaped the critical attention of pathologists for several years, owing to the fact that in some ways, upon superficial examination, it may frequently appear somewhat similar to the common scab of potatoes. However, upon careful comparison no one can have the slightest doubt but that this dry core or pitting is entirely different from the form of injury produced by *Oospora scabies* Thaxter, later considered by Lutman and Cunningham as identical with *Actinomyces chromogenus* Gasperini.

In greenhouse experiments Morse and Shapovalov<sup>2</sup> observed that this pitting was closely associated with *Rhizoctonia*. They found *Rhizoctonia* filaments within these pits and that infection apparently originated at the lenticels. However, their conclusions were based entirely upon general observations and upon the fact that the filaments of the fungus were constantly associated with all stages of the development of the diseased areas. No critical study was made of the relationship of the supposed parasite to the host tissues.

It is the purpose of this paper to describe this form of disease more fully and to present evidence which tends to show that *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.) is largely, if not entirely, responsible for this type of injury.

There are two phases of this disease that should be noted. First, the stage that on superficial examination might be mistaken for common scab. Second, a stage showing a canal formation which might be confused with the injury caused by the wireworm. The first stage is most generally noticeable where the infection is less than 3 mm. in diameter. However, there are exceptions to this.

The fungus enters at the lenticels and works its way down into the tuber without much external disturbance. The original outer cortex, being left more or less intact, forms a roof over the diseased area. The definite boundary and dark-brown color of the area suggest a form of scab. The interior granular mass of hyphæ, broken-down cells, and

<sup>1</sup> Rolfs, F. M. Potato failures. A second report. Colo. Agr. Exp. Sta. Bul. 91, p. 11. 1904.

<sup>2</sup> Morse, W. J., and Shapovalov, Michael. Loc. cit.

starch grains all remain in position, forming a dry "plug" and suggesting the name "dry core."

The second phase of this type of injury is most often found in the older stages in the progress of the disease or where the infected area reaches a diameter greater than 3 mm. This stage is most likely to be found when the potato tubers have reached maturity and the disease has run its course, or after the tubers have been stored. Owing to a drying out and shrinkage of tissues, a pit or canal is formed in the center of the affected area. Doubtless in harvesting and storing the tubers this pitting is furthered by parts of this granular material being shaken out or loosened. There is no doubt that the greater part of this pitting, particularly in cases where the disease has penetrated deeply into the flesh, has been attributed in Maine to the work of the wireworm. However, close observation even by the layman will readily show a great difference between these two types of injury.

The diseased areas are approximately circular in outline and at the surface vary in size from that of a lenticel to 6 or 7 mm. in diameter. They usually extend into the flesh of the tuber to a depth equal to or somewhat greater than the diameter. The dry core or pit thus formed gradually tapers off, forming a somewhat rounded end, very seldom becoming pointed. The majority of these pits are proportioned and shaped quite like a thimble, but some of them have a tendency to become longer and more slender. In such cases a dry, roughly cylindrical core may penetrate the flesh of the tuber for some distance. A casual observer might readily attribute this form of injury to insect attacks. In a few cases the pit has taken a more or less horizontal direction in reference to the surface of the tuber, and, as shown in Plate 27, *F*, two of these pits have joined together some little distance below the surface of the tuber.

Infection takes place in lenticels. Even from the very earliest stages the infected areas become slightly darker and sunken from the surrounding tissues. The mycelium of the fungus seems to travel equally in all directions; thus, as time passes, the infected area becomes a larger and larger circle dark brown in color. Surrounding the mature pit there is a very definite line of demarcation separating the diseased tissue from the healthy. In fact, this line is so definitely laid down that one has little or no difficulty in inserting the point of a knife and lifting out the whole core, leaving a clear-cut cavity in the healthy flesh of the tuber. By boiling tubers affected in this manner, the majority of the cores will come out clean with the peeling when it is removed. Plate 28, *E-I*, shows a group of these cores that came out with the peeling after the tuber has been boiled for 20 minutes. It will be seen (Pl. 29, *A*) that this division line is formed by three or four layers of compact suberized cells that have been laid down by the potato to prevent further penetration of its tissues by the fungal hyphæ. This leather-like lining of the

pit, once thoroughly established, effectively stops further progress of the fungus. No fungal filaments have ever been found penetrating this lining, and none have been found in the healthy tissues surrounding any of these pits. The evidence shows that the size of the pit is determined by the rate of suberization of cells sufficiently far enough in advance to effectively block further progress of the fungus. From very earliest infection, when the diseased area is scarcely more than an enlarged lenticel, the host cells always show more or less suberization three or four cells in advance of the deepest situated fungal filament. This being uniformly the case, together with the fact that the cells seem to die and lose their contents some distance ahead of the fungus, has suggested the possibility of a toxic substance being secreted by the hyphæ.

Microtome sections varying from 8 to 15  $\mu$  in thickness have been made from all stages of the "dry core" from the earliest, showing only the infected lenticel, to the age when the diseased area has ceased to enlarge, some as great as 6 mm. in diameter. In every case *Rhizoctonia* mycelium has been found. These infected areas are remarkably free from secondary fungi. In characteristic cases there is no histological evidence whatever of the presence of any other fungus. This, coupled with the fact that pure cultures of *Rhizoctonia* have been obtained repeatedly from these infections, seems to present a very strong case against *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.).

What appeared on the surface of a tuber as an enlarged lenticel is shown in section in Plate 30, *B*. After sectioning and staining this material, it was a matter of surprise to find *Rhizoctonia* hyphæ so readily, and in all sections made of this area. It will be seen from the illustrations that the host cells have already begun to suberize four cells in advance of the fungal hyphæ. No doubt this displacement and ragged edges of the normal corky cells of the cortex has been emphasized on account of their being washed and torn out in the process of fixing and cutting. However, it is believed that this will not prove misleading to anyone who has had experience in working with materials of this kind. The gradual progress of the fungus and the disintegration of the host cells, in what might be termed the second step, is shown in figure *C* (Pl. 30). This affected area was slightly over 1 mm. in diameter. By comparison with figure *A* (Pl. 30), which is a section of a normal portion of this same tuber, the results produced by the invading fungus may readily be seen. As the infection spreads and the fungal filaments become more and more abundant, this disintegration process goes on until the interior of the core is converted into a mass of broken-down cells, hyphæ, and free starch, thus giving an appearance, when magnified, similar to Plate 30, *D*. The hyphæ in this case will be seen to be of the sclerotia-forming type, as compared with the earlier infection stages or with the pure-culture hyphæ of figure *F* (Pl. 30). In a few cases the writer had the good fortune to get sections in which the outer cortex had remained more or less



intact, thus preventing the loss of the granular mass inside the core. Sclerotia-forming hyphæ were very abundant and in some instances, such as figured in Plate 30, *D*, cells more or less broken down were completely filled and apparently held in shape by the compact mass of mycelium. The mycelium of this character seemed to adhere to or follow the cell walls to a great extent.

The writer has seen little evidence of actual cell-wall penetration by the hyphæ. While Plate 29, *B*, seems to show that the fungal filaments can penetrate the walls, yet from the numerous sections made of diseased tissue practically no other cases could be found, although diligent search was made. The host cells are always found to have been killed, their walls suberized and more or less collapsed, quite some distance in advance of the fungal hyphæ. A falling apart and a separation of the cell walls suggest some kind of action upon the middle lamellæ. All of this action, taken together with the natural mechanical breakdown due to drying out and shrinkage, soon converts the interior of the core into a dry granular mass partially held together by the mycelium of the fungus.

Drayton,<sup>1</sup> in his study of *Rhizoctonia* lesions upon the potato stem, says:

Individual hyphae were found running longitudinally and sometimes obliquely in the cells of these tissues and in the intercellular spaces:

This statement is made with reference to the vascular bundles and pith of the stem, and, although he does not actually say that cell-wall penetration takes place, yet one might infer that this does occur. It is believed, however, that this does not necessarily conflict with the present findings. Within the tuber the hyphæ find quite a different situation and seemingly have considerable difficulty in penetrating the cell walls.

#### SUMMARY

(1) Direct mention of this form of potato injury was first made in Maine. If reference elsewhere has been made to it, the descriptions were not adequate to warrant connection with this form of injury.

(2) Two phases of the injury are worthy of notice: One whose external appearance somewhat resembles scab and which extends as a dry core into the flesh of the tuber; another in which the shrinkage of tissues has formed a pit or canal in the center of the infected area, frequently suggesting wireworm injury.

(3) Careful histological studies of all stages in the progress of the injury invariably show the presence of *Rhizoctonia* hyphæ.

(4) Pure cultures of *Rhizoctonia* have repeatedly been obtained from the interior parts of the diseased areas.

(5) Evidence shows that the host cells die and lose their contents, and the walls suberize and are more or less broken down several cells in

<sup>1</sup> Drayton, F. L. The *Rhizoctonia* lesions on potato stems. In *Phytopathology*, v. 5, no. 1, p. 61. 1915.

advance of the fungal filaments. This might lead one to suspect that part of the action is due to a toxin that is secreted by the fungus.

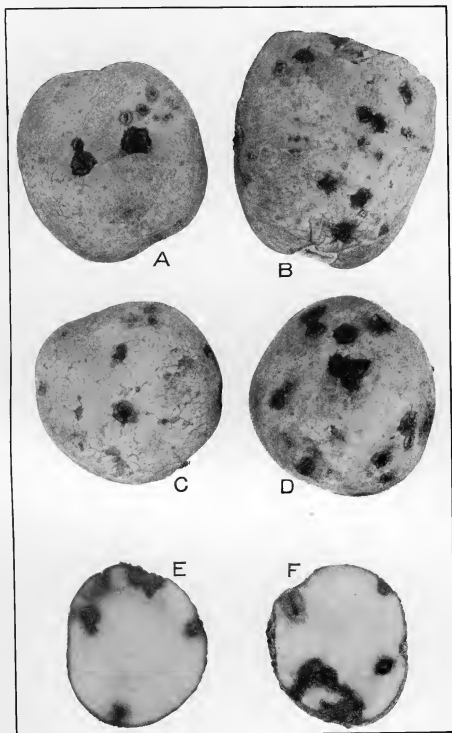
(6) Actual cell-wall penetration by the *Rhizoctonia* hyphæ apparently may occur, but this seems to be the exception rather than the rule.

#### PLATE 27

A-D.—Various stages of the "dry core" of potato tubers.

E-F.—Cross sections of a tuber badly affected with *Rhizoctonia*. Figure F shows two of the cores joined together. Compare with figure E of Plate 28, which illustrates a similar core taken bodily out of a boiled potato.





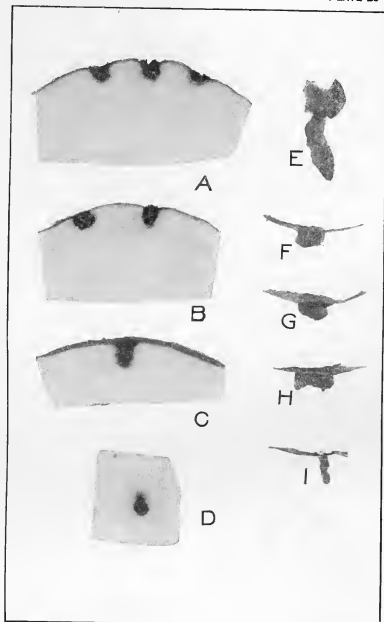


PLATE 28

A-C.—A longitudinal section through some of the cores of affected potato tubers. Note the dry, granular mass still inside of the darkly outlined pits.

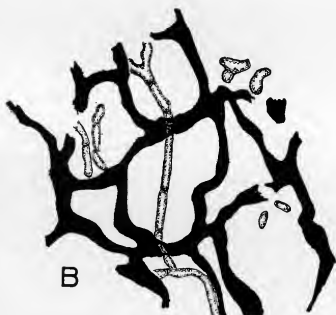
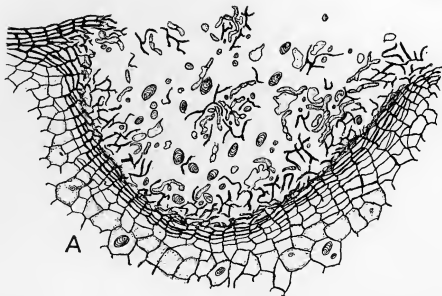
D.—Transverse section of a core.

E-I.—“Dry cores,” giving a good idea of their size and shape. These were lifted out of a boiled potato.

PLATE 29

A.—A pit slightly over 2 mm. in diameter, showing broken down cells, free starch, and fungal hyphæ. Note the layer of suberized cells arising from the outer cortex and forming a lining to the pit.

B.—Fungal hyphæ apparently penetrating the cell walls.



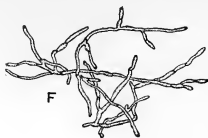
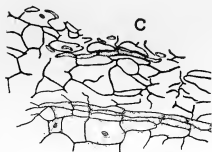
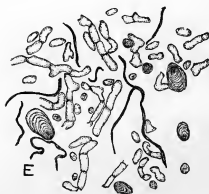
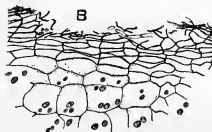
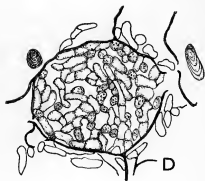
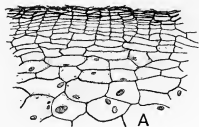




PLATE 30

- A.—The normal cortex and inner tissues of a potato tuber.
- B.—An enlarged lenticel, showing a very early stage of infection.
- C.—Fungal filaments and broken down cells in an infected lenticel slightly over 1 mm. in diameter.
- D.—A host cell highly magnified showing the interior filled with fungal hyphæ.
- E.—The granular contents of one of the pits as it appears under the microscope.
- F.—Rhizoctonia hyphæ from a pure culture isolated from the interior part of a dry core.

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